Regulation of Cerebral Blood Flow with Emphasis on Age and Environment

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Summary

Regulation of cerebral blood flow (CBF) is crucial for maintenance of oxygen and nutrient supply to the brain to assure proper function. One of the major regulators of CBF is partial pressure of arterial CO_2 ($PaCO_2$). CBF is attenuated with age both at rest and during exercise and concomitantly an age-associated reduction in $PaCO_2$ has been demonstrated which may partly contribute to the decline in CBF with aging. Additionally, cerebrovascular reactivity to CO_2 has been reported to be decreased with advancing age and thus may contribute to age-related reduction in CBF. As with advancing age, high altitude exposure challenges cerebral perfusion and has also been reported to affect cerebrovascular reactivity to CO_2, however, outcomes are inconsistent.

The purpose of the present PhD project was twofold. 1) To elucidate the role of CO_2 on the regulation of cerebral blood flow during exercise with aging. To better understand alterations in CBF with aging during exercise, cerebrovascular reactivity to CO_2 was assessed in young and elderly humans and CO_2 was added to inspiration during exercise in combination with CBF determination. 2) To investigate the influence of high altitude on cerebrovascular reactivity to CO_2. For this purpose cerebrovascular reactivity to CO_2 was assessed in young and healthy volunteers during acute exposure and acclimatization to high altitude in a controlled setting.

The two independent studies included in the present PhD thesis revealed the following: 1) Supplemental CO_2 reduced the age-associated decline in middle cerebral artery mean velocity by 50 %, suggesting that partial pressure of arterial CO_2 is a major component in the age-related reduction in cerebral blood flow during exercise. 2) In response to high altitude acclimatization cerebrovascular reactivity to CO_2 is increased, which may serve to maintain precise regulation of CBF in this environment.

Zusammenfassung

Um die Sauerstoff- und Nährstoffversorgung des Gehirns, und damit dessen einwandfreie Funktion zu gewährleisten, ist eine funktionierende Regulation der Hirndurchblutung unerlässlich. Einer der wichtigsten Regulatoren der Hirndurchblutung ist der arterielle Partialdruck von CO_2 ($PaCO_2$). Mit zunehmendem Alter sinkt die Hirndurchblutung in Ruhe und während Belastung. Gleichzeitig wurde ein erniedrigter $PaCO_2$ im Alter nachgewiesen, welcher zumindest teilweise der Grund für die altersabhängige Reduktion der Hirndurchblutung darstellen könnte. Zusätzlich wurde eine Abnahme der Reaktivität der Hirngefässe auf CO_2 gemessen, welche daher als weitere mögliche Ursache für die altersbedingte Abnahme der Hirndurchblutung gehandelt werden kann. Ähnlich wie bei zunehmendem Alter stellt der Aufenthalt in der Höhe eine Herausforderung für die Regulation der Hirndurchblutung dar. Eine veränderte Reaktivität der Hirngefässe auf CO_2 wurde zwar dokumentiert, jedoch wiedersprechen sich die Ergebnisse.

Das vorliegende Dissertationsprojekt beinhaltet folgende zwei grundlegende Zielsetzungen: 1) Das Erläutern der Rolle von CO_2 in der Regulation der Hirndurchblutung während körperlicher Belastung im Alter. Um die Änderungen der Hirndurchblutung im Alter besser zu verstehen, wurde während körperlicher Belastung CO_2 zur Einatmungsluft hinzugefügt und gleichzeitig die Hirndurchblutung gemessen. 2) Das Untersuchen des Einflusses der Höhe auf die Reaktivität der Hirngefässe auf CO_2. Dafür wurde die Reaktivität der Hirngefässe auf CO_2 in jungen gesunden Studienteilnehmern während akuter und chronischer Höhenexposition mit kontrollierten Rahmenbedingungen bestimmt.

Folgende Ergebnisse stammen aus den zwei unabhängigen Studien, welche das vorliegende Dissertationsprojekt beinhaltet: 1) Die Zugabe von CO_2 führte zu einer 50 % Verminderung der altersbedingten Reduktion der Geschwindigkeit der mittleren Hirnarterie während Belastung. Dies unterstreicht den Einfluss von $PaCO_2$ auf die altersabhängige Reduktion der Hirndurchblutung. 2) Höhenakklimatisation führte zu einer erhöhten Reaktivität der Hirngefässe auf CO_2 und könnte so eine präzise Regulation der Hirndurchblutung in der Höhe fördern.

Acknowledgments

I would like to express my gratitude to Prof. Dr. Carsten Lundby who has supported me throughout my PhD studies and provided me with great possibilities in his productive research laboratory at the University of Zurich and thus enabled me to pursue my scientific goals. Additionally, I would like to thank Prof. Dr. Martin Wolf, PD Dr. Johannes Sarnthein, Prof. Dr. Johannes van Lieshout and Prof. Dr. Reto Huber for their support and being part of my PhD committee. I am also grateful to all lab members for their help and support, specifically to Mario Widmer, Stefanie Keiser and Saskia Gehrig for their friendship, laughs and company during long working days. I would like to thank all co-authors for their outstanding collaboration throughout the process of the present studies. I would also like to thank my family and Severin Nowak for their love, encouragement and continuous support. Finally, I would like to thank all study participants that volunteered in the studies.

1. Introduction

Regulation of cerebral blood flow

The human brain accounts for merely ~ 2 % of the body weight, however receives ~ 15 % of the total cardiac output and utilizes ~ 20 % of the oxygen taken up by the human organism at rest (Lassen 1959). Together with its limited capacity for substrate storage (Brown and Ransom 2007) this underlines the high energy demand of the brain. Hence, a precise regulation of cerebral blood flow (CBF) is crucial for maintenance of constant nutrient and oxygen supply to the brain.

Arterial blood supply to the brain is shared between two pairs of large arteries, the right and left internal carotid and vertebral arteries. The vertebral arteries join distally to form the basilar arteries and branches from the vertebral and basilar artery supply the cerebellum and the brainstem. Additionally, the basilar artery joins the two internal carotid arteries to form the circle of Willis which is the origin of the three main intracerebral arteries; the anterior, middle, and posterior. These arteries then progressively divide into smaller arteries, arterioles and capillaries. Pial arteries run along the surface of the brain, arterioles enter the brain tissue and capillaries finally supply blood to the corresponding regions (Cipolla 2009). In this PhD project middle cerebral artery mean velocity ($MCAv_{mean}$) was assessed using transcranial Doppler ultrasound (TCD) as a surrogate for CBF.

Substantial reductions in CBF quickly lead to unconsciousness and if maintained, brain damage and death occur (Van Lieshout et al. 2003). Likewise, severe elevations in cerebral perfusion pressure may result in hemorrhage, breakthrough edema and stroke (Pires et al. 2013).

Meticulous control of CBF involves a wide range of regulatory mechanisms that together work to ensure optimal oxygen and nutrient delivery (Willie et al. 2014). Principal regulators of CBF are partial pressure of arterial CO_2 ($PaCO_2$, (Ide et al. 2003), mean arterial pressure (MAP, Lucas et al. 2010), cerebral metabolism (Belanger et al. 2011) and the autonomic nervous system (Hamner et al. 2010).

Even minor reductions in oxygen supply to the brain, as induced during strenuous exercise or exercise at high altitude, have been demonstrated to result in reduced exercise performance due to failure in neural drive from the motor cortex (Goodall et al. 2012). Additionally, chronic cerebral hypoperfusion, e.g. associated with aging, has been associated with cognitive impairment (Bertsch et al. 2009).

In the present PhD thesis the focus will be on CO_2 as it is one of the major regulators of CBF. The aim is to elucidate the role of CO_2 during exercise with aging as well as the influence of high altitude on the cerebrovasculature by assessing cerebrovascular reactivity to CO_2.

Cerebral blood flow and CO_2

Half a century ago, low and high tensions of CO_2 were demonstrated to affect the cerebrovasculature (Kety and Schmidt 1948). Since then, CO_2 has been accepted to be one of the major regulators of CBF. Elevations in $PaCO_2$ (hypercapnia) lead to vasodilation (Figure 1), whereas decreases in $PaCO_2$ (hypocapnia) facilitate vasoconstriction of the cerebrovasculature (Ide et al. 2003). CBF increases by approximately 3 - 5 % and 2 - 3 % per increase and decrease from the resting value in mmHg of CO_2, respectively (Poulin et al. 1996; Ide et al. 2003; Willie et al. 2012).

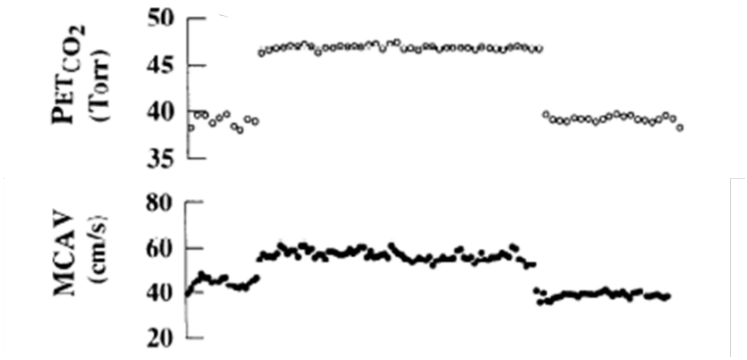

Figure 1. Middle cerebral artery velocity (MCA_V) in response to changes in end-tidal partial pressure of CO_2 ($PetCO_2$). (Modified from (Poulin et al. 1996)).

These sensitivities are unique to the cerebrovasculature compared with the peripheral vasculature (Ainslie et al. 2005) and are also used as a measure of cerebrovascular function (Willie et al. 2011), called cerebrovascular reactivity to CO_2 (CVR). Additionally, the CO_2 induced vasodilation and vasoconstriction are involved in a homeostatic function by either removing CO_2 from the cerebral tissue and thus increasing pH or restraining CO_2 in the cerebral tissue leading to a reduction in pH, respectively (Chesler 2003).

Although the effects of CO_2 on the cerebral vessels have been assessed for decades, the underlying mechanisms leading to the changes in CBF are not fully elucidated. It is debated whether $PaCO_2$, pH, or both are responsible for the changes in CBF. Inducing changes in pH while keeping $PaCO_2$ maintained did not lead to alterations in CBF (Harper and Bell 1963; Berne et al. 1981). A few years later, Kontos et al. (Kontos et al. 1977) demonstrated the importance of extracellular pH in the vasoactivity of cerebral vessels. Vasodilation and vasoconstriction in response to a reduction and elevation in extracellular pH, respectively, have been observed in cultured cerebral vascular muscle cells (Apkon et al. 1997). Furthermore, the mechanism causing a dilation in response to hypercapnia likely involves nitric oxide (Iadecola 1992).

In the present studies CO_2 is used to manipulate CBF to investigate the role of CO_2 in the regulation of CBF during exercise with aging as well as to assess cerebrovascular function in young and older healthy volunteers and in response to high altitude.

1.1 Age, aerobic fitness and cerebral perfusion during exercise: Role of carbon dioxide

Cerebral blood flow and exercise with age

During exercise CBF increases with increasing exercise intensity until approximately 60 % of maximal oxygen uptake (VO_2max) and thereafter declines towards resting levels (Hellström et al. 1996; Fisher et al. 2008; Fisher et al. 2012; Marsden et al. 2012). The regulation of CBF during exercise is complex and underlies an integrative regulation. Elevated cerebral metabolism, blood pressure, cardiac output and $PaCO_2$ possibly play a role in the elevation in CBF in response to exercise (Querido and Sheel 2007; Ogoh and Ainslie 2009). The decrease in cerebral perfusion during high exercise intensities is likely the consequence of a hyperventilation induced reduction in $PaCO_2$ and thus leading to a vasoconstriction. This indicates CBF being mainly regulated by $PaCO_2$ as CBF decreases despite further elevations in exercise intensity and cerebral metabolism (Ogoh and Ainslie 2009). Furthermore, adding CO_2 to inspiration and thus counteracting the drop in $PaCO_2$ abolishes the decline in CBF observed at higher exercise intensities (Olin et al. 2011; Siebenmann et al. 2013). The gradual decline in CBF with exercise intensity above a certain threshold also reduces cerebral oxygenation. This has been speculated to lead to centrally mediated fatigue and thus may limit exercise performance (Nielsen et al. 1999; Goodall et al. 2012).

With healthy aging, CBF at rest, independent of the applied method to determine CBF, is reduced compared with young and healthy individuals (Kety 1956; Melamed et al. 1980; Ainslie et al. 2008; Demirkaya et al. 2008; Galvin et al. 2010; Fisher et al. 2012; Liu et al. 2012; Murrell et al. 2012; Zhu et al. 2013). During exercise CBF follows the above described pattern, however, in comparison with young volunteers CBF is reduced with increasing age (Fisher et al. 2012). Additionally, CVR has been reported to be decreased in older individuals (Bakker et al. 2004; Flück et al. 2014) and may contribute to the age-associated reduction in CBF. Yet, controversial data exists (Galvin et al. 2010; Zhu et al. 2013). Generally, the physiological mechanisms leading to a decline in CBF with aging remain uncertain. Possible mechanisms for the age-related decrease in CBF include increased arterial stiffness (Zhu et al. 2011; Flück et al. 2014) and global brain

atrophy (Fjell et al. 2009), although the latter has been argued not to contribute to the age-related decline in CBF (Chen et al. 2011). Additionally, the concomitantly occurring and age-dependent decrease in $PaCO_2$ at rest (Fisher et al. 2012) and during exercise (Fisher et al. 2012; Marsden et al. 2012) may be involved in the age-associated reduction in cerebral perfusion (Figure 2).

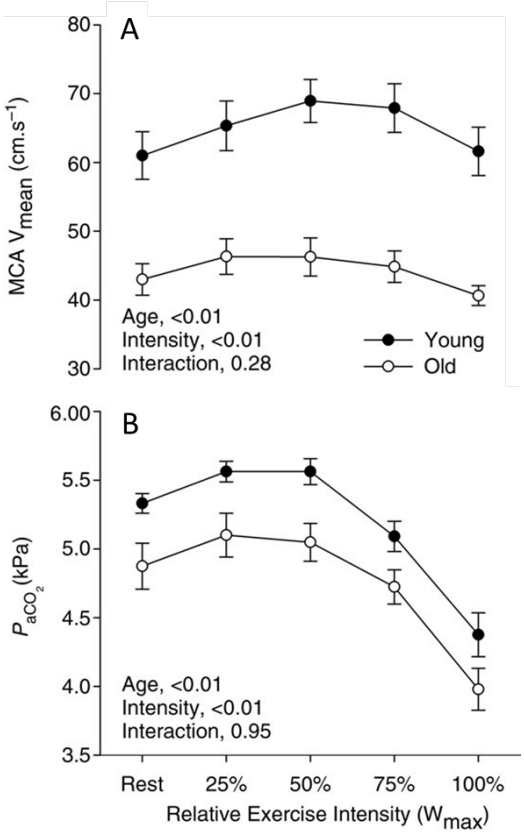

Figure 2. Middle cerebral artery mean velocity ($MCAv_{mean}$, A) and arterial partial pressure of CO_2 ($PaCO_2$, B) at rest and during exercise in young and older individuals. (Modified from (Fisher et al. 2012)).

Cerebral blood flow and aerobic fitness

Beneficial effects of an increased aerobic fitness on the cerebrovasculature have been demonstrated (Ainslie et al. 2008; Bailey et al. 2013; Thomas et al. 2013), however, this has not been universally observed (Barnes et al. 2013; Zhu et al. 2013). The influence of aerobic fitness was expressed by the association of resting CBF or CVR with VO_2max. Ainslie et al. (Ainslie et al. 2008) observed an elevated resting CBF in active vs. sedentary individuals over a wide age range while

contradictory results derive from studies with a study cohort with a narrow age range (Barnes et al. 2013; Zhu et al. 2013). CVR has been reported to be increased after a 12-week exercise training (Murrell et al. 2012). On the other hand, compared with sedentary elderly, hypercapnic CVR was not elevated in master athletes (Zhu et al. 2013).

Study aim

The aims of the first study included in the present PhD thesis were: 1) to determine the effect of age on hypercapnic CVR compared with healthy young individuals, 2) to establish the importance of the age-related drop in $PaCO_2$ on $MCAv_{mean}$ by administering supplemental CO_2 to inspiration during exercise, 3) to examine the effects of aerobic fitness on hypercapnic CVR and the age-related decrease in $MCAv_{mean}$ by comparing aged and young individuals that are aerobically trained or untrained and 4) to investigate whether maintaining $MCAv_{mean}$ and thereby cerebral oxygenation would lead to an improvement in exercise capacity in young and older individuals.

1.2 Cerebrovascular reactivity increases with acclimatization to 3454 m

Cerebral blood flow and high altitude

High altitude exposure leads to a decrease in arterial partial pressure of O_2 (PaO_2) and $PaCO_2$. In comparison to $PaCO_2$, the cerebrovasculature is less sensitive to changes in PaO_2, with vasodilation only occurring when PaO_2 declines below ~ 60 mmHg (Gupta et al. 1997). With acute exposure to high altitude, the hypoxic vasodilatory effect facilitates an elevation in CBF. However with acclimatization to altitude this is counteracted by the hypoxic ventilatory response which causes a further reduction in $PaCO_2$ and thus CBF declines towards sea-level values (Severinghaus et al. 1966; Møller et al. 2002). CVR may intuitively be expected to be decreased in response to the ventilatory induced reduction in $PaCO_2$ in order to preserve CBF. Yet, several studies have assessed CVR in response to hypoxic exposure (Jensen et al. 1996; Jansen et al. 1999; Poulin et al. 2002; Ainslie and Burgess 2008; Fan et al. 2010; Lucas et al. 2011; Villien et al. 2013; Fan et al. 2014; Ogoh et al. 2014; Rupp et al. 2014) and the outcomes are highly controversial, ranging from increased (Jensen et al. 1996; Poulin et al. 2002; Fan et al. 2010; Fan et al. 2014), unchanged (Jansen et al. 1999; Villien et al. 2013; Rupp et al. 2014) to reduced (Ainslie and Burgess 2008; Lucas et al. 2011; Ogoh et al. 2014) CVR (Figure 3 and 4). These differences possibly derive in part from inconsistencies in study protocols and methods applied. Briefly, the CVR tests have been conducted in different background oxygen levels (Fan et al. 2014;Rupp et al. 2014;Villien et al. 2013), at varying time points of altitude exposure (Lucas et al. 2011) and, additionally, only a few studies have taken changes in blood pressure into account when assessing CVR (Fan et al. 2014). Furthermore, most studies have been conducted under conditions similar to a mountain expedition (Jansen et al. 1999; Ainslie and Burgess 2008; Fan et al. 2010; Lucas et al. 2011) including a trekking ascent over several days, board and lodging, which contrast markedly with subject's usual environment and habits, all being potential confounding factors.

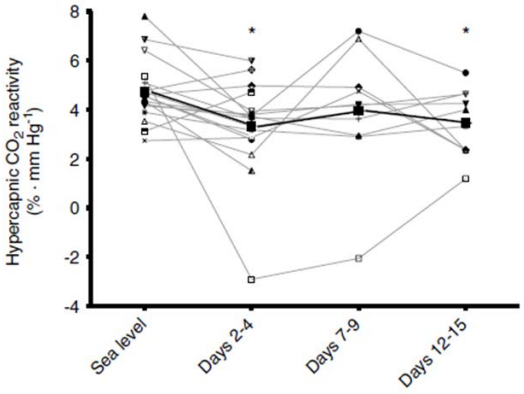

Figure 3. Individual middle cerebral artery velocity ($MCAv_{mean}$) responses during hypercapnia at sea level and after 2-4, 7-9 and 12-15 days at high altitude.* P<0.05 compared to sea level. (Modified from (Lucas et al. 2011)).

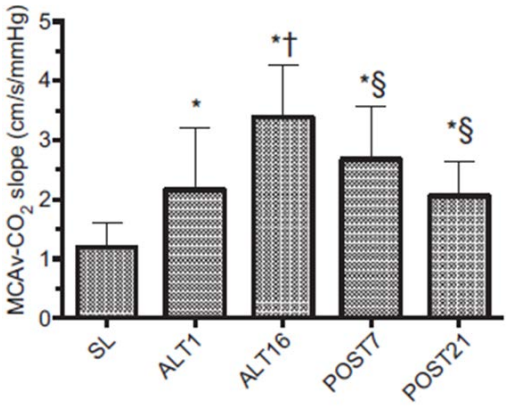

Figure 4. Changes in steady-state estimates of cerebrovascular responsiveness to CO_2 at sea level (SL), 1 (ALT1) and 16 (ALT16) days and after 7 (POST7) and 21 (POST21) days of reexposure to 5260 m. * P<0.05 compared to SL. † P<0.05 compared to ALT1. § P<0.05 compared to ALT16. (Modified from (Fan et al. 2014)).

Study aim

The aim of the second study included in the present PhD thesis was to overcome the above mentioned limitations by performing a study at the Jungfraujoch research station (3454 m) where living conditions are comparable to subject's daily life and allow for measurements immediately after a two hour train ascent. Additionally, hypo- and hypercapnic CVR were assessed in background hypoxia and normoxia at sea level and then again after exactly 30 min, 3 and 22 days of hypoxic exposure.

2. Methods

The methods used in the studies included in the present PhD thesis will be briefly introduced, however in detail information can be found in the published manuscripts.

2.1 Cerebral blood flow

In 1945, Kety and Schmidt modified the inert gas method, previously used to measure cardiac output, to also determine CBF. The method is based on the Fick principle (Fick 1870), where flow can be determined as the ratio between the uptake of an inert gas per unit time and the arterio-venous inert gas difference across the organ. Kety and Schmidt have used the inert gas nitrous oxide to determine CBF which was introduced to the arterial blood via the lungs (Kety and Schmidt 1948), but also other freely diffusible tracers have been used (e.g. Xenon, Hydrogen). This method is considered the gold standard in research, however it also encompasses limitations. Briefly, the measurement is time consuming and does not allow to observe dynamic changes in CBF, it is also very invasive and it assesses only a global measure of CBF (Willie et al. 2011).

The introduction of Transcranial Doppler Ultrasound (TCD) brought the advantage of assessing dynamic changes in CBF as it has a high temporal resolution, in addition to being a non-invasive and a rather inexpensive method (Aaslid et al. 1982). Through the temporal window, where the skull is thin and thus insonation of the three large intracranial arteries are possible, a pulse of ultrasound is transmitted using an ultrasound probe. The emitted ultrasound is scattered by the moving erythrocytes and the frequency shift (Doppler shift) of the detected ultrasound signal by the receiver is proportional to the velocity of the blood. It has to be considered though that TCD assesses velocity and not flow in absolute terms, as the vessel diameter of the insonated artery remains unknown. In order to asses CBF by using TCD an unchanged vessel diameter is assumed (Aaslid et al. 1982). Several studies have demonstrated a constant vessel diameter (Poulin and Robbins 1996; Serrador et al. 2000; Verbree et al. 2014), however, recently small changes in vessel diameter have been observed in response to extreme increases in $PaCO_2$ or decreases in PaO_2 (Wilson et al. 2011; Willie et al. 2012; Verbree et al. 2014). Consequently, when using TCD to assess $MCAv_{mean}$ limitations have to be discussed. Nevertheless, the assessment of blood velocity

using TCD has been accepted as a reliable measure of CBF. Its small size and fixation to a headband enables continuous measuring during experimental protocols such as cycling (Willie et al. 2011).

For the studies included in this PhD thesis the MCA was insonated using a 2 MHz probe (Doppler Box, DWL, Sipplingen, Germany). $MCAv_{mean}$ was assessed continuously through the experiments with the ultrasound probe held in place with a headgear.

2.2 Inspired gas manipulations and respiratory parameters

By wearing a mask, covering nose and mouth, or breathing through a mouthpiece with the nose occluded, respiratory parameters were assessed breath by breath (Cosmed Quark b2, Rome, Italy). In order to manipulate partial pressure of end tidal CO_2 ($PetCO_2$) and O_2 ($PetO_2$), CO_2 was added to inspiration and ambient air was diluted with nitrogen or O_2 via a mixing chamber (Altitrainer, SMTEC, Nyon, Switzerland). Continuous recording of breath by breath respiratory parameters enabled an accurate control of desired CO_2 or O_2 levels. A reduction in $PetCO_2$ was achieved through voluntary hyperventilation by the study participants. In the first study included in the present PhD project, resting $PaCO_2$ and $PaCO_2$ during the hypercapnic CVR test were estimated from $PetCO_2$ using the Peebles' calculation (Peebles et al. 2007). During exercise $PaCO_2$ was estimated using the Jones' equation (Jones et al. 1979).

2.3 Cerebrovascular reactivity to CO_2

Combining the two above mentioned methods enables the assessment of CVR. As mentioned before, CO_2 is a major regulator of CBF and therefore CVR assesses the ability of the cerebrovasculature to react to changes in $PetCO_2$. The reactivity of the cerebrovasculature also serves as a surrogate of cerebrovascular function (Willie et al. 2011). In the following studies, we have used step protocols to elevate $PetCO_2$ and voluntary hyperventilation to induce a reduction in $PetCO_2$. Simultaneously, $MCAv_{mean}$ was measured and reactivity was calculated as either an absolute or a relative change in $MCAv_{mean}$ per increase in $PetCO_2$. Due to the known effects of MAP on CBF as well as changes in MAP induced by alterations in $PetCO_2$ (Claassen et al. 2007; Ainslie and Duffin 2009), it is important to concomitantly measure MAP. MAP was recorded

continuously and noninvasively via finger photopletysmography (Nexfin, BMEYE B.V, Amsterdam, Netherlands). Moreover cerebrovascular conductance (CVC) can be calculated ($MCAv_{mean}$ / MAP) as well as CVC CVR to take changes in MAP into account (Claassen et al. 2007; Regan et al. 2014).

2.4 Exercise testing

In order to determine study volunteer's maximal workload (Wmax) and VO_2max an incremental exercise test on a cycle ergometer was performed (Monark E 839, Vaarberg, Sweden). According to the study participants achieved Wmax an individualized exercise protocol consisting of two absolute workloads (60, 100 W) and four relative workloads (25, 50, 75, 100 % Wmax) was generated. This allowed for comparison at the same absolute and relative workloads.

2.5 Cerebral and muscle oxygenation

Near infrared spectroscopy (NIRS; Invos-5100c, Covidien, Mansfield, MA) was used to assess cerebral and muscle tissue oxygenation. NIR light is emitted into the tissue, during its penetration through the tissue, before reaching the detector, NIR light is absorbed and scattered. According to the changes of light intensity from its emission to its detection an index for tissue oxygenation derives (Boushel et al. 2001). NIRS sensors were placed on the forehead and the vastus lateralis muscle to assess cerebral and muscle tissue oxygenation, respectively.

3. Manuscripts

3.1 Age, aerobic fitness and cerebral perfusion during exercise: role of carbon dioxide

3.2 Cerebrovascular reactivity increases with acclimatization to 3454 m

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Age, aerobic fitness, and cerebral perfusion during exercise: role of carbon dioxide

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Flück D, Braz ID, Keiser S, Hüppin F, Haider T, Hilty MP, Fisher JP, Lundby C. Age, aerobic fitness, and cerebral perfusion during exercise: role of carbon dioxide. *Am J Physiol Heart Circ Physiol* 307: H515–H523, 2014. First published June 20, 2014; doi:10.1152/ajpheart.00177.2014.—Middle cerebral artery mean velocity ($MCAv_{mean}$) is attenuated with increasing age both at rest and during exercise. The aim of this study was to determine the influence of the age-dependent reduction in arterial P_{CO_2} ($PaCO_2$) and physical fitness herein. We administered supplemental CO_2 (CO_2 trial) or no additional gas (control trial) to the inspired air in a blinded and randomized manner, and assessed middle cerebral artery mean flow velocity during graded exercise in *1*) 21 young [Y; age 24 ± 3 yr (±SD)] volunteers of whom 11 were trained (Y_T) and 10 considered untrained (Y_{UT}), and *2*) 17 old (O; 66 ± 4 yr) volunteers of whom 8 and 9 were considered trained (O_T) and untrained (O_{UT}), respectively. A resting hypercapnic reactivity test was also performed. $MCAv_{mean}$ and $PaCO_2$ were lower in O [44.9 ± 3.1 cm/s and 30 ± 1 mmHg (±SE)] compared with Y (59.3 ± 2.3 cm/s and 34 ± 1 mmHg, $P <$ 0.01) at rest, independent of aerobic fitness level. The age-related decreases in $MCAv_{mean}$ and $PaCO_2$ persisted during exercise. Supplemental CO_2 reduced the age-associated decline in $MCAv_{mean}$ by 50%, suggesting that $PaCO_2$ is a major component in the decline. On the other hand, relative hypercapnic reactivity was neither influenced by age ($P = 0.46$) nor aerobic fitness ($P = 0.36$). Although supplemental CO_2 attenuated exercise-induced reduction in cerebral oxygenation (near-infrared spectroscopy), this did not influence exercise performance. In conclusion, $PaCO_2$ contributes to the age-associated decline in $MCAv_{mean}$ at rest and during exercise; however exercise capacity did not diminish this age effect.

brain blood flow; middle cerebral artery; old; transcranial Doppler

CEREBRAL BLOOD FLOW (CBF) is meticulously regulated to ensure an adequate perfusion of the brain. With exercise middle cerebral artery mean velocity ($MCAv_{mean}$; a surrogate measure of CBF) is increased until ~60% of maximal oxygen uptake ($\dot{V}O_{2max}$) but thereafter declines toward resting levels (10, 20, 28). In young healthy individuals this drop in cerebral perfusion is likely the consequence of a hyperventilation facilitated reduction in $PaCO_2$ and hence augmented cerebral vasoconstriction (39). Accordingly, administration of CO_2 to the inspired air during exercise abolishes the decrease in $MCAv_{mean}$ (44, 45), and during vigorous exercise $MCAv_{mean}$ is regulated by $PaCO_2$ and only to a lesser extent influenced by cerebral metabolism, mean arterial pressure (MAP), cardiac output, or sympathetic nerve activity (35).

Compared with young healthy individuals a reduced CBF (24) and $MCAv_{mean}$ has consistently been reported in the aged population both at rest (2, 7, 9, 15, 25, 27, 49) and during exercise (9, 10, 28, 32). Although a reduced $MCAv_{mean}$ response in aged humans is observed with exercise, its pattern follows that of young individuals, i.e., an initial increase which is then followed by a decline as the exercise intensity becomes intense (9, 10, 28, 32). The regulating mechanisms for the reduction in CBF with age remain uncertain and global brain atrophy (11), decreased neuronal activity (29), increased arterial stiffness (12, 50), and reduced cerebrovascular reactivity (12, 22) have all been proposed as important factors. In addition, the concomitantly occurring and age-dependent decrease in $PaCO_2$ at rest (9) and during exercise (9, 28) may be involved in the age-associated reduction in cerebral perfusion. This however has not been tested experimentally, and is one aim of the present study. An age-related reduction in CO_2 reactivity could also diminish the CBF response to exercise. The effect of aging on CO_2 reactivity is not clear, however, as hypercapnic reactivity has been demonstrated to be either unchanged (15, 24, 42), reduced (4, 22, 48), or even elevated (49) with increasing age. One reason for the discrepancies could be related to the use of humans over a narrow age range, and with varying degrees of physical fitness. Thus a further aim related to this study was to quantify CO_2 reactivity and to investigate its association to the decrease in $MCAv_{mean}$ with age.

A high aerobic fitness level appears to attenuate the age-related decline in resting CBF (39) and $MCAv_{mean}$ (1–3, 46), although this has not been universally observed (4, 49). Whether fitness is a factor affecting CBF during exercise

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remains unclear. Moreover, aerobic fitness may affect hypercapnic cerebrovascular reactivity (4, 32, 49), although these findings are also contradictory as increased (3, 32), unchanged (49) and decreased (46) hypercapnic reactivity has been reported in aerobically trained individuals. Again potential influencing factors for the widespread results could include differences in age and fitness levels across studies.

Accordingly the purpose of the present study was fourfold: *1*) to determine the effect of age on hypercapnic reactivity compared with healthy young individuals, *2*) to establish the importance of the age-related drop in $PaCO_2$ on $MCAv_{mean}$ by administering supplemental CO_2 to the inspiration during exercise, *3*) to examine the effects of physical fitness on hypercapnic reactivity and the age-related decrease in $MCAv_{mean}$ by comparing aged and young individuals that are aerobically trained or untrained, and *4*) to investigate whether maintaining $MCAv_{mean}$ and thereby cerebral oxygenation would lead to an improvement in exercise capacity in young and older individuals. We hypothesized that supplementing the inspired air with CO_2 during exercise would reduce the age-related reduction in $MCAv_{mean}$. Second, we hypothesized that elderly individuals with superior aerobic fitness would possess a higher $MCAv_{mean}$ than their untrained peers both at rest and during exercise, and that this difference would also be accompanied by a higher hypercapnic reactivity. Finally we expected that improvements in $MCAv_{mean}$ elicited by CO_2 supplementation would not lead to an improved exercise capacity.

METHODS

All experimental protocols and procedures conformed to the Declaration of Helsinki and were approved by the ethical committee of the Swiss Federal Institute of Technology Zürich (EK 2013-N-17). Prior to participation, a detailed verbal and written explanation of the study was provided, and written informed consent to take part in this study was obtained from each participant. Subjects were screened by means of a general health questionnaire to identify any history or symptoms of cardiovascular (e.g., hypertension), pulmonary, metabolic, or neurological disease or use of medications. We recruited 21 young (Y_{T+UT}) males and 24 older (O_{T+UT}) males to participate in the study. Based on an evaluation of their medical history and a physical examination, as well as a 12-lead resting ECG assessing abnormality of the heart, seven older subjects were excluded from participating in the study. Consequently, 21 Y_{T+UT} [24 ± 3 yr (±SD)] and 17 O_{T+UT} (66 ± 4 yr) completed the study. Subject groups were also divided into trained (T) and untrained (UT) groups independent of the type of training performed, first by self-assessment and then confirmed by their achievement in the maximal incremental exercise test (43) during their preliminary visit to the laboratory. Consequently 11 young trained ($\dot{V}O_{2max}$: 65.6 ± 1.0 $ml \cdot kg^{-1} \cdot min^{-1}$; Y_T), 10 young untrained (49.6 ± 1.8 $ml \cdot kg^{-1} \cdot min^{-1}$; Y_{UT}), 8 older trained (40.5 ± 2.5 $ml \cdot kg^{-1} \cdot min^{-1}$; O_T), and 9 older untrained (29.6 ± 1.3 $ml \cdot kg^{-1} \cdot min^{-1}$; O_{UT}) individuals participated in the study (Table 1).

During a preliminary visit to the laboratory, subjects were familiarized with the exercise tests and the study set-up. On the second visit to the laboratory a hypercapnic reactivity test plus two maximal incremental exercise tests were performed, one without and one with additional CO_2 to inspiration. Between subjects the tests were conducted at different times of the day; however, within-subject comparison data were assessed at the same time of the day. Subjects were requested to abstain from strenuous physical activity for 24 h, and alcohol and caffeine for 12 h prior to experimental sessions. Monitoring procedures associated with medical safety during exercise were performed in real-time by a physician and included a 12-lead ECG, oscillometric noninvasive blood pressure measurements with a 3 min monitoring interval and a ST elevation check of I-aVF, V1-V6, in a 1-min interval. Termination criteria for the exercise test were systolic blood pressure above 220 mmHg or a 10-mmHg drop below baseline, ST anomalies, arrhythmias, and symptoms such as dizziness, syncope, or cyanosis. Posttest monitoring was for 15 min if asymptomatic and if BP and ECG reached baseline values. No test participants were excluded on the basis the above investigations.

Experimental measures. $MCAv_{mean}$ was assessed using transcranial Doppler ultrasonography (Doppler Box, DWL, Sipplingen, Germany) with a 2 MHz probe placed over the right temporal window, prepared with ultrasound gel. The probe was held in place with a snug-fitting headgear. Mean arterial pressure (MAP) was recorded continuously and noninvasively via finger photoplethysmography (Nexfin, BMEYE B.V, Amsterdam, Netherlands) and heart rate (HR) was assessed by a monitor belt (Cosmed Quark b2, Rome, Italy). $MCAv_{mean}$ and MAP were sampled at 1,000 Hz and stored for offline analysis (LabChart 7 Pro v7.3.5 and Powerlab, ADInstruments, Bella Vista, NSW, Australia).

Table 1. *Age, weight, height, BMI, MAP, absolute and relative $\dot{V}O_{2max}$, $PaCO_2$, $MCAv_{mean}$, and absolute, relative and CVC CO_2 reactivity at rest in young and older, trained and untrained individuals*

	Young		Old		*P* Value		
	Trained (n = 11)	Untrained (n = 10)	Trained (n = 8)	Untrained (n = 9)	Age	Training	Interaction
Age, yr	22 ± 0	25 ± 1	65 ± 0	67 ± 1	< 0.01	0.06	0.78
Weight, kg	71.0 ± 2.3	78.8 ± 2.7	72.1 ± 2.5	82.3 ± 3.9	0.46	< 0.01	0.69
Height, m	1.82 ± 0.02	1.81 ± 0.02	1.72 ± 0.01*	1.79 ± 0.02	< 0.01	0.07	0.05
BMI, kg/m^2	21.4 ± 0.5	23.9 ± 0.8	24.2 ± 0.9	25.4 ± 1.1	0.02	0.04	0.44
MAP, mmHg	91.3 ± 1.8	92.3 ± 1.8	92.0 ± 3.4	94. ± 2.8	0.67	0.60	0.85
Wmax, W	388 ± 16	318 ± 12	238 ± 10	216 ± 17	< 0.01	<0.01	0.13
Absolute $\dot{V}O_{2max}$, l/min	4.66 ± 0.14	3.88 ± 0.12	2.90 ± 0.16	2.42 ± 0.12	< 0.01	< 0.01	0.32
Relative $\dot{V}O_{2max}$, $ml \cdot min^{-1} \cdot kg^{-1}$	65.6 ± 1.0	49.6 ± 1.8	40.5 ± 2.5	29.6 ± 1.3	< 0.01	< 0.01	0.15
$PaCO_2$, mmHg	34.8 ± 0.5	32.8 ± 0.9	30.4 ± 0.8	29.4 ± 0.9	< 0.01	0.07	0.56
$MCAv_{mean}$, cm/s	56.1 ± 2.7	62.9 ± 3.3	49.7 ± 4.9	40.6 ± 2.8*	< 0.01	0.76	0.04
Absolute CO_2 reactivity, $cm \cdot s^{-1} \cdot mmHg^{-1}$	1.99 ± 0.37	1.57 ± 0.26	1.95 ± 0.36	1.82 ± 0.21	0.73	0.38	0.64
Relative CO_2 reactivity, %/mmHg	3.42 ± 0.68	2.80 ± 0.29	3.64 ± 0.36	3.32 ± 0.54	0.49	0.38	0.78
CVC CO_2 reactivity, $cm \cdot s^{-1} \cdot mmHg^{-2}$	0.016 ± 0.003	0.013 ± 0.002	0.010 ± 0.003	0.009 ± 0.001	0.06	0.47	0.71

Values are means ± SE. BMI, body mass index; MAP, mean arterial pressure; $\dot{V}O_{2max}$, maximal oxygen uptake; $PaCO_2$, estimated arterial partial pressure of CO_2; $MCAv_{mean}$, middle cerebral artery mean velocity; CVC, cerebrovascular conductance. *P* values represent ANOVA results. *$P < 0.05$ vs. young. Young trained and untrained and older trained and untrained are compared at rest.

By wearing a mask covering nose and mouth (Hans Rudolph) respiratory variables were measured breath-by-breath using a spirometer. A capillary blood sample was obtained from the right ear lobe at BL, and during exercise at 100 W, at 75% maximal workload (Wmax), and at exhaustion. Capillary P_{CO_2} ($PcapCO_2$), pH, and HCO_3^- were measured using a blood gas analyzer (ABL800, Radiometer, Copenhagen, Denmark). Cerebral and muscle tissue oxygenation (cerebral and muscle StO_2) were continuously assessed on the left forehead and right vastus lateralis muscle, respectively, by near-infrared spectroscopy (NIRS; Invos-5100c, Covidien, Mansfield, MA).

Hypercapnic reactivity test. Hypercapnic reactivity test was performed by adding CO_2 to the inspired air (Altitrainer, SMTEC, Nyon, Switzerland) with subjects in a semisupine position. After a 10-min resting phase, $PaCO_2$ (estimated from end-tidal P_{CO_2}) was recorded for 3 min following which the CO_2 reactivity test protocol was undertaken. The protocol consisted of three steps of 120 s: step 1 ($PaCO_2$ = +1.5 mmHg above resting values), step 2 ($PaCO_2$ = +6.5 mmHg above resting values), and recovery ($PaCO_2$ = +1.5 mmHg above resting values). Maintaining $PaCO_2$ at 1.5 mmHg above resting values facilitates $PaCO_2$ control (21) and reduces breath-to-breath variability in cerebral blood flow velocity (19).

Exercise test. During the preliminary visit to the laboratory each subject performed a maximal incremental exercise test on a cycle ergometer (Monark E 839, Varberg, Sweden) to determine their Wmax and $\dot{V}O_{2max}$ using a protocol starting with a warm-up period of 5 min at 100 W (Y_T), 150 W (Y_T), 20 W (O_{UT}), or 50 W (O_T). Thereafter the workload was increased every minute by 30 W (Y_T, Y_{UT} and O_T) or 20 W (O_{UT}) until exhaustion.

On the second visit to the laboratory subjects completed two incremental exercise tests in a blinded and randomized manner without (control trial) and with supplemental inspired CO_2 (CO_2 trial). The trials were separated by at least 90 min to limit any potential carryover effect (41). The workload selected during the exercise protocol was based on the Wmax achieved during the maximal incremental exercise test performed on the first visit. Both the control trial and the CO_2 trial followed the same individualized protocol consisting of two absolute workloads (60 W, 100 W) and four relative workloads (25% Wmax, 50% Wmax, 75% Wmax, and 100% Wmax). The first four workloads were set to last 3 min each, the fifth workload for 2 min, and the final workload (100% Wmax) was performed until exhaustion. The duration that the subjects were able to sustain 100% Wmax was used as a measure of exercise performance. This protocol design allows comparison between groups at the same absolute and relative exercise intensities. Prior to each exercise test a 2 min resting measurement on the cycle ergometer (BL) was conducted. The control trial was performed without the addition of supplemental inspired CO_2 whereas $PaCO_2$ was continuously monitored in the CO_2 trial and when $PaCO_2$ dropped below 40 mmHg CO_2 was added to the inspired air preventing the exercise-induced drop in $PaCO_2$.

Data analysis and calculations. Data were recorded continuously. Resting $PaCO_2$ and $PaCO_2$ during the hypercapnic reactivity test was estimated from end-tidal P_{CO_2} ($P_{ET}CO_2$) by the equation (37):

$$\text{estimated } PaCO_2 = 2.367 + 0.884 \times P_{ET}CO_2$$

During the exercise tests $PaCO_2$ was estimated from $P_{ET}CO_2$ by the equation (23):

$$\text{estimated } PaCO_2 = 5.5 + 0.9 \times P_{ET}CO_2 - 0.0021 \times V_T$$

where tidal volume (VT) is in milliliters.

Resting $MCAv_{mean}$, MAP, and $PaCO_2$ were averaged over the last minute of the resting phase prior to the hypercapnic reactivity test. Baseline, absolute, and relative exercise intensity values represent an average over 1 min whereas the "last 20 s" time point represents an average of the last 20 s of each exercise trial. This was chosen to reduce the chance of missing the changes in the measured parameters at maximal exercise shortly before exhaustion. Cerebrovascular conductance (CVC) was calculated as $MCAv_{mean}$ divided by MAP. CO_2 reactivity was calculated as absolute and relative changes in $MCAv_{mean}$ divided by the increase in $PaCO_2$. Additionally CVC CO_2 reactivity was calculated as CVC changes divided by the increase in $PaCO_2$.

Statistics. Comparisons of resting values were made using a two-way ANOVA with the main factors being age (young vs. old) and training status (trained vs. untrained). For each the absolute and relative exercise intensities a separate four-way ANOVA was used for exercise variable comparisons, where the main factors were age, training status, intensity (BL, 60 W, 100 W and BL, 25%, 50%, 75%, 100% Wmax, last 20 s) and condition (control vs. CO_2 trial). Tukey's range test was applied for post hoc analysis. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using SAS Enterprise Guide (4.3, SAS Institute, Cary, NC).

RESULTS

All 38 volunteers completed the study. Independent of the participant's physical fitness level there was a main age effect between the Y_{T+UT} and the O_{T+UT} (Table 1). Briefly, resting MAP was similar in the two groups. $PaCO_2$ and $MCAv_{mean}$ were higher in Y_{T+UT} compared with O_{T+UT}. Furthermore, an interaction for $MCAv_{mean}$ between age and training was found and post hoc analysis revealed an age difference within the untrained groups.

Maximal workload and oxygen uptake with exercise. Wmax achieved by Y_{T+UT} was 35% higher (354 ± 13 vs. 227 ± 11 W, $P < 0.01$) than in O_{T+UT}. Within the age groups, trained participants reached 18% and 9% (Y_{T+UT} and O_{T+UT}) higher Wmax compared with the untrained participants (388 ± 10 vs. 318 ± 13 W, $P < 0.01$, Y_T vs. Y_{UT}; 238 ± 10 vs. 216 ± 19 W, $P < 0.01$, O_T vs. O_{UT}). Absolute and relative $\dot{V}O_{2max}$ are presented in Table 1. $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), and respiratory exchange ratio (RER) are presented in Table 2.

Hypercapnic reactivity test. $PaCO_2$ was increased ($P < 0.01$) from step 1 to step 2 in Y_{T+UT} (38.1 ± 0.5 to 42.2.0 ± 0.6 mmHg) and O_{T+UT} (34.5 ± 0.7 to 38.5 ± 0.7 mmHg). Concomitantly MAP was augmented by 3.3 ± 0.8 and 5.3 ± 0.8 mmHg in Y_{T+UT} and O_{T+UT}, respectively. Hypercapnic reactivity data (Table 1) are presented in absolute and relative $MCAv_{mean}$ as well as absolute CVC changes to 1 mmHg increase in $PaCO_2$. Neither of these differed between the age groups and trained vs. untrained study volunteers. CVC CO_2 reactivity, however, tended ($P = 0.06$) to be slightly lower with age.

$PaCO_2$, $MCAv_{mean}$, MAP, and CVC in the control vs. the CO_2 exercise trial. Figure 1 illustrates $PaCO_2$, $MCAv_{mean}$, MAP, and CVC data from the control and CO_2 trial for Y_{T+UT} (Y_T and Y_{UT} pooled) and O_{T+UT} (O_T and O_{UT} pooled). In the control trial, $PaCO_2$ was elevated with increasing exercise intensity until ~75% Wmax and thereafter decreased in both age groups. In accordance with the lower $PaCO_2$ at BL, $PaCO_2$ during exercise (control trial) was also lower ($P < 0.01$) in O_{T+UT} compared with Y_{T+UT}. CO_2 was administrated to inspiration during exercise in the CO_2 trial to keep $PaCO_2$ above 40 mmHg (Y_{T+UT}, 42.6 ± 0.4 mmHg; O_{T+UT}, 45.2 ± 0.3 mmHg). Analysis of values at absolute exercise intensities revealed an interaction of age, condition, and intensity ($P < 0.01$, Fig. 1*A*). Post hoc analysis demonstrated lower $PaCO_2$ values in the control trial of the O_{T+UT} compared with O_{T+UT} CO_2 trial and Y_{T+UT} control and CO_2 trial at 60 and 100 W. A main effect of age ($P < 0.01$) and an interaction between intensity and condition ($P < 0.01$) was observed within the relative exercise intensities data. At 75%, 100% Wmax and last

Table 2. *Oxygen uptake, carbon dioxide production, and respiratory exchange ratio during exercise in the control trial in young and older, trained and untrained individuals*

	Young		Old		*P* Values			
	Trained (n = 11)	Untrained (n = 10)	Trained (n = 8)	Untrained (n = 9)	Age	Training	Intensity	Interactions
			$\dot{V}O_2$, *ml/min*					
60 W	1,587 ± 75	1,563 ± 36	1,424 ± 94	1,319 ± 80	< 0.01	0.51	< 0.01	> 0.27
100 W	2,059 ± 76	2,099 ± 75	1,896 ± 81	1,757 ± 99				
25 % Wmax	1,994 ± 95	1,830 ± 65	1,442 ± 116	1,196 ± 87	< 0.01	< 0.01	< 0.01	< 0.01
50 % Wmax	3,112 ± 129	2,839 ± 127	2,156 ± 134*	1,785 ± 111*				
75 % Wmax	4,080 ± 174	3,482 ± 134	2,656 ± 138*	2,279 ± 131*				
100 % Wmax	4,459 ± 141	3,692 ± 138	2,732 ± 235*	2,429 ± 156*				
			$\dot{V}CO_2$, *ml/min*					
60 W	1,197 ± 55	1,215 ± 55	1,177 ± 85	1,099 ± 68	0.12	0.81	< 0.01	> 0.15
100 W	1,685 ± 70	1,844 ± 80	1,655 ± 75	1,588 ± 99				
25 % Wmax	1,614 ± 70	1,553 ± 61	1,172 ± 114	971 ± 70	< 0.01	< 0.01	< 0.01	< 0.01
50 % Wmax	2,667 ± 82	2,654 ± 120	1,925 ± 124*	1,615 ± 81*				
75 % Wmax	3,849 ± 143	3,604 ± 130	2,591 ± 134*	2,181 ± 101*				
100 % Wmax	5,231 ± 212	4,296 ± 190	2,897 ± 245*	2,550 ± 125*				
			RER					
60 W	0.76 ± 0.02	0.78 ± 0.01	0.82 ± 0.01*	0.83 ± 0.02*	< 0.01	0.04	< 0.01	< 0.01
100 W	0.82 ± 0.02	0.88 ± 0.02†	0.87 ± 0.01	0.90 ± 0.02†				
25 % Wmax	0.81 ± 0.01	0.85 ± 0.01	0.81 ± 0.02	0.81 ± 0.02	< 0.01	0.07	< 0.01	<0.01
50 % Wmax	0.86 ± 0.02	0.94 ± 0.02	0.89 ± 0.01	0.91 ± 0.02				
75 % Wmax	0.95 ± 0.02	1.04 ± 0.02	0.98 ± 0.02	0.96 ± 0.02				
100 % Wmax	1.17 ± 0.02	1.16 ± 0.03	1.06 ± 0.02*	1.06 ± 0.03*				

Values are means ± SE. $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio. Wmax, maximal workload. *P* values represent ANOVA results. *$P < 0.05$ vs. young, †$P < 0.05$ vs. trained.

20 s, $PaCO_2$ was elevated in the CO_2 trial compared with the control trial in both age groups.

Figure 1*B* presents $MCAv_{mean}$ values during the control and CO_2 trial for both age groups. Throughout exercise $MCAv_{mean}$ in the O_{T+UT} was lower compared with Y_{T+UT}. $MCAv_{mean}$ was higher at 100% Wmax and the last 20 s in the CO_2 trial compared with the control trial in Y_{T+UT} and O_{T+UT}.

MAP responses to exercise are presented in Fig. 1*C*. Training status did not show an effect on MAP response during absolute or relative exercise intensities in both age groups ($P > 0.58$). CVC for the control and CO_2 trial and both age groups is presented in Fig. 1*D*. At absolute and relative exercise intensities a main effect of age was apparent. In agreement with $MCAv_{mean}$, CVC was also increased at 100% Wmax and the last 20 s in the CO_2 trial compared with the control trial in both age groups.

Ventilation and exercise performance in the control vs. the CO_2 exercise trial. Ventilation ($\dot{V}E$) was elevated with increasing exercise intensity ($P < 0.01$); however, it was not different between Y_{T+UT} and O_{T+UT} at BL, 60 W, and 100 W (14.7 ± 0.6 vs. 14.6 ± 0.7, 34.7 ± 0.8 vs. 34.7 ± 1.5 and 47.1 ± 1.3 vs. 51.4 ± 2.0 l/min, $P = 0.22$). Analysis of $\dot{V}E$ during relative exercise intensities revealed an interaction between age and intensity ($P < 0.01$), and at 75% Wmax (96.8 ± 3.1 vs. 73.8 ± 2.6 l/min), 100% Wmax (134.5 ± 3.4 vs. 87.8 ± 2.8 l/min), and last 20 s (140.0 ± 3.6 vs. 90.6 ± 3.1 l/min) $\dot{V}E$ was elevated in Y_{T+UT} vs. O_{T+UT}. A main effect of condition was only apparent when absolute intensities [e.g., 100 W in O_{T+UT}: 48.9 ± 2.4 vs. 53.9 ± 3.2 l/min, control vs. CO_2 trial ($P < 0.01$)] were evaluated, whereas there was no main effect of condition when relative exercise intensities were assessed ($P = 0.52$).

The duration at 100% Wmax was lower in the O_{T+UT} compared with the Y_{T+UT} ($P < 0.01$), independent of their fitness level ($P = 0.49$). Additionally both age groups sustained a shorter duration at 100% Wmax during the CO_2 trail (Y_T, 106 ± 11 s; Y_{UT}, 83 ± 11 s; O_T, 50 ± 11 s; O_{UT}, 48 ± 11 s) compared with the control trial (Y_T, 116 ± 12 s; Y_{UT}, 85 ± 11 s; O_T, 61 ± 10 s; O_{UT}, 87 ± 16 s; $P = 0.01$).

Trained vs. untrained study volunteers. Figure 2 illustrates the same data as in Fig. 1 except that age groups are divided into a trained (Y_T and O_T) and untrained group (Y_{UT} and O_{UT}) according to their $\dot{V}O_{2max}$ (Table 1). A main effect of training status was not evident for $MCAv_{mean}$, MAP, CVC, and $\dot{V}E$ at absolute or relative exercise intensities. $PaCO_2$ during relative exercise intensities, however, demonstrated a main effect of training status. An interaction between intensity and training status was observed, along with an interaction between condition and training status. $PaCO_2$ values during the control trial in both age groups were lower in the untrained vs. trained volunteers. Training status did not exert an effect on $PaCO_2$ when compared at absolute exercise intensities.

Relative changes in $PaCO_2$, $MCAv_{mean}$ and CVC during control and CO_2 trial. Percent changes from baseline are similar to that observed for the absolute values (Fig. 1) except that the magnitude of the age-related difference in the $MCAv_{mean}$ and $PaCO_2$ response to exercise was diminished. The supplemental CO_2 given to inspiration resulted in a greater increase in CO_2 in the O_{T+UT} compared with the Y_{T+UT} during exercise at the same absolute intensity.

Capillary blood samples. Due to an insufficient volume of blood being obtained from some of the participants these data are derived from $n = 13$ (8 Y_{T+UT} and 5 O_{T+UT}). $PcapCO_2$ values are in agreement with the estimated $PaCO_2$ values from the spirometer ($r = 0.76$, $P < 0.01$). Baseline pH did not differ between Y_{T+UT} (7.40 ± 0.01) and O_{T+UT} (7.41 ± 0.01, $P = 0.80$). At exhaustion pH in the Y_{T+UT} was 7.27 ± 0.02 and 7.27 ± 0.02 for control and CO_2 trial, respectively, and in O_{T+UT} pH was 7.34 ± 0.01 and 7.33 ± 0.01. A main effect of

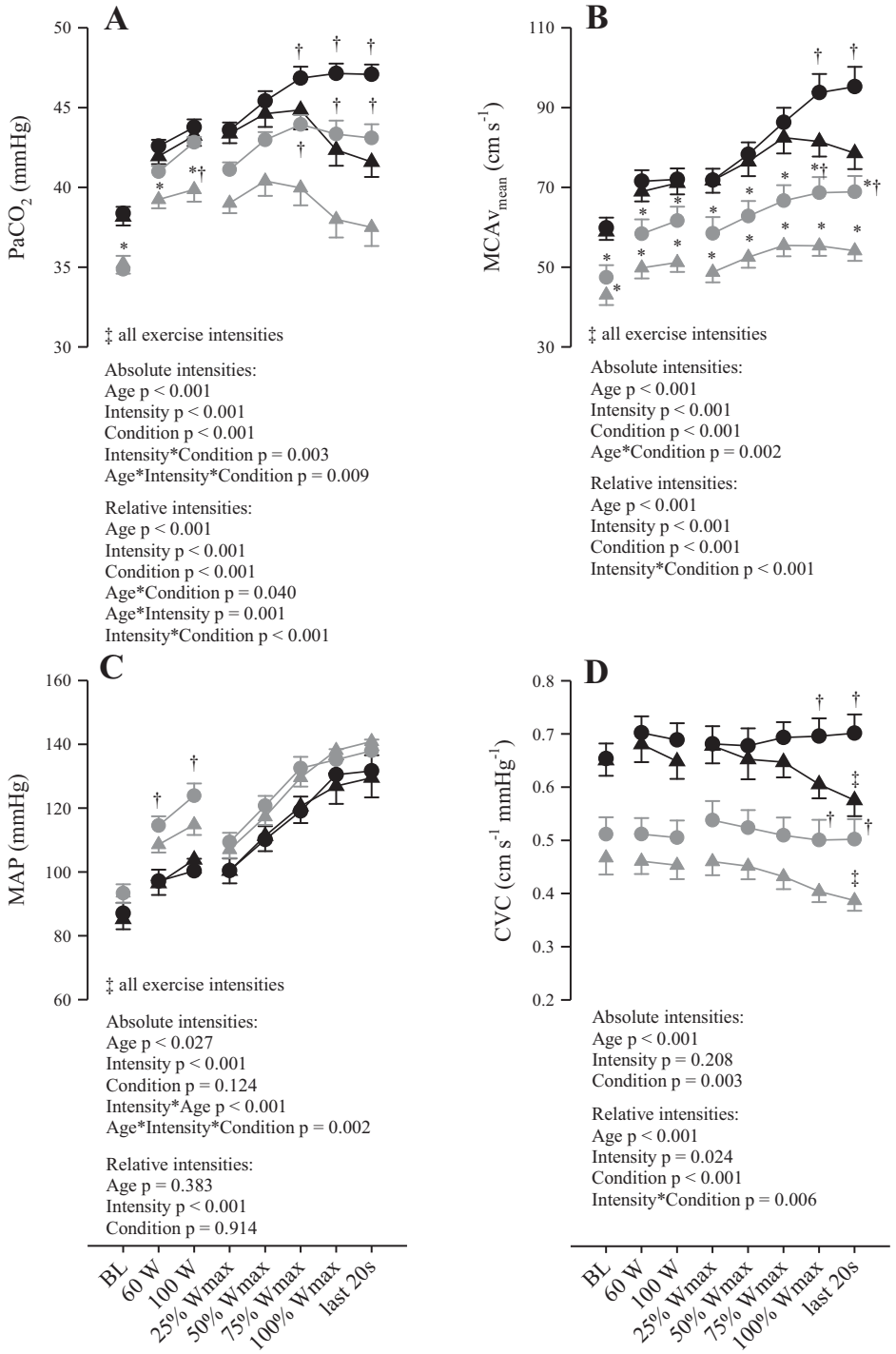

Fig. 1. Arterial P_{CO_2} ($PaCO_2$; mmHg; *A*), mean middle cerebral artery velocity ($MCAv_{mean}$; cm/s; *B*), mean arterial pressure (MAP; mmHg; *C*), and cerebrovascular conductance (CVC; $cm \cdot s^{-1} \cdot mmHg^{-1}$; *D*) in young (black symbols) and old (gray symbols) with (circles) and without (triangles) administered CO_2. Measurements were completed at baseline (BL) and throughout graded maximal exercise. Wmax, maximal workload. Values are means ± SE. *P* values represent ANOVA results. *$P < 0.05$ vs. young, †$P < 0.05$ vs. control trial, ‡$P < 0.05$ vs. baseline.

age ($P < 0.01$) was observed in pH, but there was no main effect of either condition ($P = 0.14$) or training status ($P = 0.44$). No main effects of condition ($P = 0.99$) or training ($P = 0.23$) were found for HCO_3^-, but there was an interaction between age and intensity ($P < 0.01$). At exhaustion HCO_3^- was lower in Y_{T+UT} vs. O_{T+UT} in the control trial and CO_2 trial (18.2 ± 0.9 vs. 20.7 ± 0.3 mmol/l and 18.3 ± 0.6 vs. 20.5 ± 0.5 mmol/l, $P < 0.01$).

Cerebral and vastus lateralis muscle oxygenation. Cerebral StO_2 was increased in response to supplemental CO_2 whereas muscle StO_2 was unaffected (Fig. 3). Changes in cerebral and muscle oxygenation followed a similar pattern in both age groups; however, greater decreases in muscle oxygenation were observed in the young group when relative exercise intensities were compared.

DISCUSSION

In support of the study aims the major findings include: *1*) confirmation of the age-associated decrease in $PaCO_2$ and

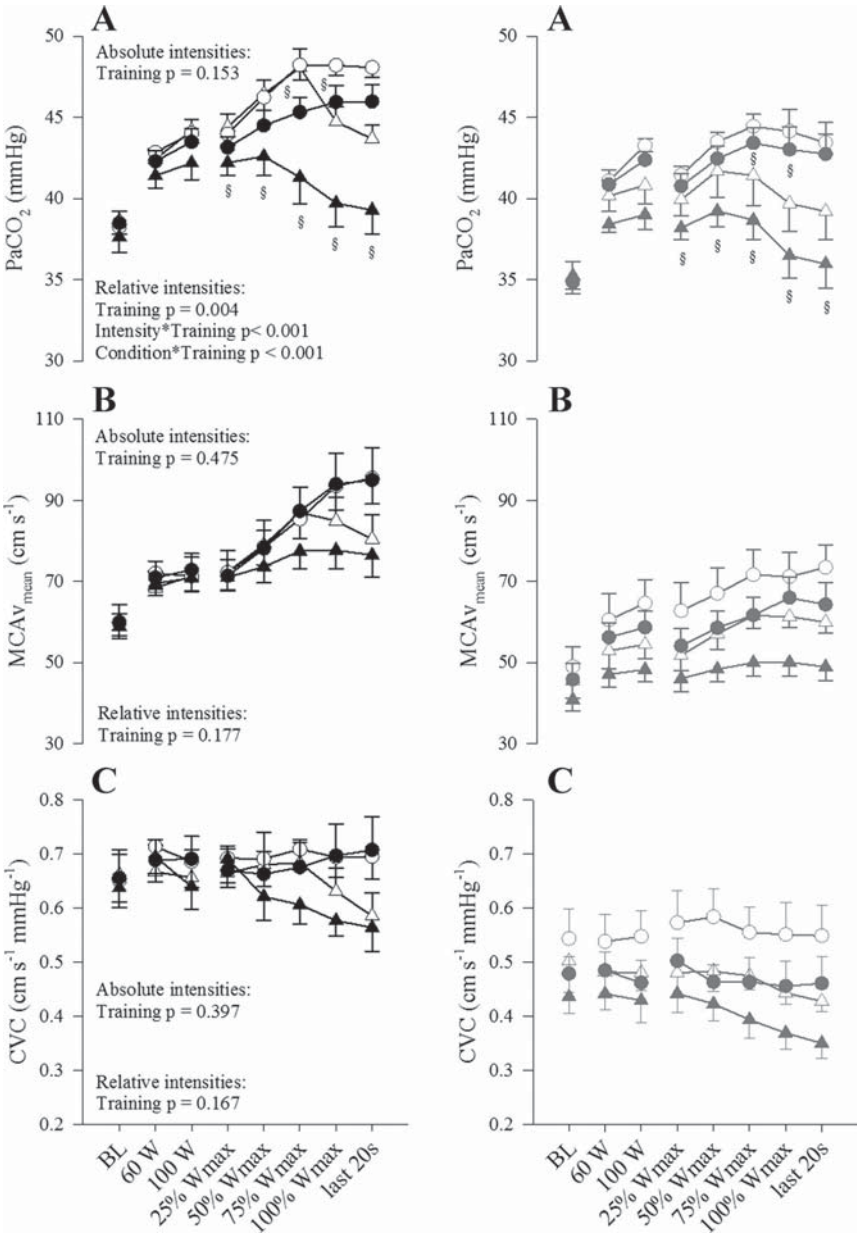

Fig. 2. $PaCO_2$ (mmHg; *A*), $MCAv_{mean}$ (cm/s; *B*), and CVC ($cm \cdot s^{-1} \cdot mmHg^{-1}$; *C*) in young (left panel) and old (right panel) trained (open symbols) and untrained (filled symbols) subjects with (circles) and without (triangles) CO_2 administered. Values are means ± SE. *P* values represent ANOVA results. §$P < 0.05$ vs. trained.

$MCAv_{mean}$ at rest and during exercise; *2*) hypercapnic cerebrovascular reactivity is not altered with increased age; *3*) the experimentally induced increase in $PaCO_2$ abolished ≈50% of the age-related reduction in $MCAv_{mean}$ during exercise; *4*) aerobic fitness in young and older humans does not influence $MCAv_{mean}$ at rest or during exercise; and *5*) improvements in cerebral oxygenation by CO_2 administration do not lead to improved exercise performance in either young or older study participants.

Influence of age on resting $PaCO_2$ and $MCAv_{mean}$. The reduction in resting $MCAv_{mean}$ with age observed in the present study is in agreement with studies assessing CBF using transcranial Doppler ultrasonography (7, 9, 15, 32, 49), the Kety-Schmidt technique (25), and arterial spin labeling MRI (27). Possible mechanisms for the age-related decrease in CBF include decreased neuronal activity (29), increased arterial stiffness (50), reduced cerebrovascular reactivity (22), and global brain atrophy (11), although the latter has been argued not to contribute to the age-related decline in CBF (6). Reduced $PaCO_2$ has also been associated to the age-related decrease in $MCAv_{mean}$ (9) and in agreement herewith we observed that $PaCO_2$ and $MCAv_{mean}$ are both lower in the older study participants. Others, however, have not demonstrated such a relationship (15, 28, 32, 49), although three of four of these studies reported that $PaCO_2$ was 1 to 2.8 mmHg lower in the older subjects. A possible explanation for $PaCO_2$ to be lower with age in some humans could be secondary to age-related metabolic acidosis (13). However, in a follow up study the same authors reported elevated blood acidity in the elderly, but no age-related difference in $PaCO_2$ was reported (14). Furthermore, in the present study resting capillary blood pH was not reduced with age, and does hence not support an

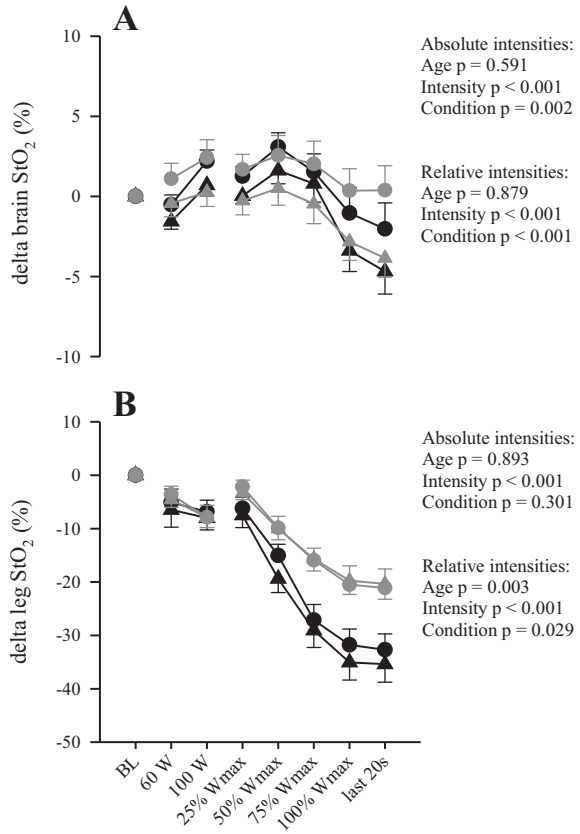

Fig. 3. Relative changes (delta) to baseline (BL) for cerebral (brain StO_2; *A*) and muscle (leg StO_2; *B*) tissue oxygenation in response to exercise performed with (circles) or without (triangles) administered CO_2 to inspiration in young (black symbols) and old (gray symbols) participants. Values are means ± SE. *P* values represent ANOVA results.

age-related acidosis, at least in the subjects participating in the present study.

$PaCO_2$ and $MCAv_{mean}$ during exercise in old and young humans. In the present study $PaCO_2$ increased gradually in the young and older groups until an exercise intensity of ≈75% Wmax was reached, and thereafter declined in agreement with previous work (10, 20). Although $MCAv_{mean}$ followed a similar pattern, the decline, however, was less apparent. Cerebrovascular conductance (CVC; $MCAv_{mean}$/MAP) exhibited a diminished elevation with increasing exercise intensity and was markedly decreased at maximal exercise (Fig. 1). This could be indicative of MAP being the dominating force increasing CBF during the early light phase of exercise (39) and the subsequent switch to $PaCO_2$ becoming the dominant regulator of CBF at higher exercise intensities.

Age did not influence the response pattern of $MCAv_{mean}$, $PaCO_2$, or CVC; however, in the older group, lower $MCAv_{mean}$, $PaCO_2$, and CVC values were observed throughout compared with the younger participants (Fig. 1). Possible mechanisms underlying this age effect remain unclear. To test the importance of $PaCO_2$ for the age-associated decrease in $MCAv_{mean}$, young and old volunteers performed an exercise trial with CO_2 administered to the inspired air. This approach has been used in different settings (8, 36, 44) and proven effective in maintaining or increasing $PaCO_2$ and $MCAv_{mean}$(36, 44). When increasing $PaCO_2$ in the aged population to values similar to those observed in the young controls (Fig. 1*A*), ≈50% of the age-related difference in $MCAv_{mean}$ was abolished (Fig. 1*B*), indicating that although a major part of the reduction in $MCAv_{mean}$ with age is related to the lower $PaCO_2$, other factors are also involved. It has been suggested that differences in $PaCO_2$ may account for 30% of the age-related decline in $MCAv_{mean}$ (1), and thus according to the present study this may be somewhat an underestimate. As $MCAv_{mean}$ and $PaCO_2$ in young and old participants present an age-related difference already at rest, the difference observed during exercise could derive from here. The percentage change in $MCAv_{mean}$ during exercise was similar in young and old and in agreement with a previous study (28), although at 50% $\dot{V}O_{2peak}$ a lower $MCAv_{mean}$ response was observed in the elderly. Similarly, the percent changes from baseline in $PaCO_2$ were comparable in young and old participants. Thus exercise does not appear to exacerbate the age-related difference in $PaCO_2$ and $MCAv_{mean}$ manifested at rest.

As $PaCO_2$ accounts for ≈50% of the age related decrease in $MCAv_{mean}$, other factors must also contribute to the decline. As mentioned decreased neuronal activity (29), increased arterial stiffness (50) and reduced cerebrovascular reactivity (4, 22, 48) are potential contributors. Cerebrovascular reactivity, which in the current study was assessed as hypercapnic reactivity, demonstrated no difference between the two groups when expressed as absolute and relative changes in $MCAv_{mean}$, in support of previous studies (15, 24). Unfortunately none of these studies determined CVC reactivity. In the present study a trend ($P = 0.06$) toward a reduced hypercapnic reactivity was evident. In contrast an increase in hypercapnic reactivity, calculated as relative and CVC changes, in elderly has been reported (49). Since no apparent explanation seems at hand for the discrepancies, the inconsistencies in hypercapnic reactivity results might arise from differences in the CBF measurement techniques, CO_2 stimulus, and age range of the study participants. In future studies blood pressure changes and therefore CVC reactivity should also be taken into consideration.

Despite having a lower $MCAv_{mean}$ this may not necessarily also lead to a lower brain oxygenation in the elderly, as a reduced oxygen delivery to the brain may be compensated for by an augmented oxygen extraction and thereby maintain cerebral oxygenation (34). Fisher et al. (9) however demonstrated similar cerebral oxygenations despite differences in cerebral perfusion between young and older individuals. In the present study brain delta oxygenation also did not differ between age groups; one limitation, however, is that we assessed tissue oxygenation index and not absolute oxy- and deoxyhemoglobin values or direct arterio-venous differences.

The influence of aerobic fitness on $MCAv_{mean}$ and hypercapnic reactivity. Physical activity has been suggested to maintain $MCAv_{mean}$ and cerebrovascular reactivity (2, 32). In the present study aerobic fitness neither in the young nor in the old group influenced resting $MCAv_{mean}$ or hypercapnic reactivity. Recent studies (4, 49) also reported a lack of an association between fitness and $MCAv_{mean}$ in young vs. older individuals. In contrast, life-long physical activity/training has shown an attenuated age-dependent decline in resting $MCAv_{mean}$ (2, 3) and CBF (2, 3, 46). When the same relative exercise intensities were compared the trained study participants displayed a higher $PaCO_2$ in the control trial, and this was independent of age. The reason could be related to the fact that trained

participants were exercising at a higher absolute workload and hence also at a higher $\dot{V}O_2$ and in most instances therefore also higher $PaCO_2$. The elevated $PaCO_2$ in trained subjects did not lead to a concomitant higher $MCAv_{mean}$ although trained study volunteers, especially the trained older, had a higher $MCAv_{mean}$ during exercise, although not reaching statistical significance ($P = 0.16$).

Several studies have investigated the influence of aerobic fitness on hypercapnic reactivity, and in agreement with our results Zhu et. al (49) demonstrated no effect of aerobic fitness on hypercapnic reactivity. As indicated with respect to the effects of age, the effects of aerobic exercise capacity on hypercapnic reactivity are inconsistent and range from increased (3) to decreased (46) reactivities in trained individuals.

The influence of cerebral oxygenation on exercise limitations. The gradual decline in CBF with exercise intensity above a certain threshold also reduces cerebral oxygenation. This has been speculated to lead to centrally mediated fatigue (17, 33). A unique feature of CO_2 supplementation is that whereas $MCAv_{mean}$ and cerebral oxygenation are increased, oxygenation of the exercising skeletal muscles remains unaffected by the intervention (Fig. 3), and hence the isolated influence of altered brain oxygenation can be assessed. In the present study a reduced decline in cerebral oxygenation did not result in a higher exercise capacity. Our results are in line with recent work (44, 45) and hence support that brain oxygenation is not a parameter of importance for fatigue development during maximal exercise. Indeed, supplemental CO_2 administered to the inspired air tended to reduce exercise performance as the duration of the 100% Wmax step was shorter during the CO_2 trial. One potential reason for the negative influence of CO_2 administration to exercise performance is a hyperventilation-induced acidification (45, 47). Previous studies have not assessed blood gas variables at maximal exercise when CO_2 was administrated. Capillary pH was unaffected by the supplemental CO_2 gas. CO_2 induced hyperventilation could direct blood flow away from the exercising muscles and support the additional work and metabolic demand of the respiratory muscles. However, ventilation did not differ between the control and CO_2 trial.

Limitations. We assessed $MCAv_{mean}$ by TCD as a surrogate for CBF. $MCAv_{mean}$ is a measure of blood flow velocity and not a flow in absolute terms. Nevertheless the two are highly correlated (5). The majority of studies have illustrated that the diameter of the MCA does not change in response to hypercapnia (16, 38). Thus assessment of blood velocity using TCD has been accepted as a reliable measure of CBF and is widely used to study age and exercise-related effects (9, 10, 28, 32). In the present study we focused on $PaCO_2$ as it is one of the major CBF regulators; however, whether it is $PaCO_2$ or in fact pH, or both, that are responsible for the hypercapnia-induced vasodilation remains uncertain. Studies manipulating arterial pH, extravascular pH, and $PaCO_2$ proposed changes in extravascular pH to be responsible for the hypercapnia- induced vasodilation (18, 26). In the present study baseline pH values did not differ between age groups, suggesting and agreeing with the above that capillary pH may not be the main factor responsible for the decrease in $MCAv_{mean}$ in older individuals. It can, however, not be ruled out that extravascular pH may have been different in the two populations and thereby at least partly involved.

Trained study volunteers were included independent of their regimen of training conducted. This could have become a problem since it is still debated whether resistance training leads to increased arterial stiffness (31) or not (40). However, since none of the subjects were predominantly engaged in resistance training this is likely not to have influenced the study outcome. Since only male volunteers were examined this also implies that the obtained data may not necessarily be valid for female although differences are not apparent (30). The $PaCO_2$ values reported in the present study are derived from $P_{ET}CO_2$; however, there are studies reporting good correlations between the two. Also in the present study the actually obtained capillary blood gases correlated well with the $PaCO_2$ estimated from the obtained $P_{ET}CO_2$.

Conclusion. In conclusion, reduced CBF was associated with a lower $PaCO_2$ at rest and during exercise in older men. Supplemental CO_2 diminished the age-related reduction in CBF by ≈50% during exercise. An elevated aerobic capacity did not lead to increased cerebrovascular health either in our young or in our older study participants. Finally, despite the maintained oxygenation during the CO_2 trial exercise performance did not improve, indicating decreased cerebral oxygenation not to be a limiting factor.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: D.F., J.P.F., and C.L. conception and design of research; D.F., I.D.B., S.K., F.H., T.H., and M.H. performed experiments; D.F. and I.D.B. analyzed data; D.F., I.D.B., J.P.F., and C.L. interpreted results of experiments; D.F. prepared figures; D.F., J.P.F., and C.L. drafted manuscript; D.F., I.D.B., S.K., F.H., T.H., M.H., J.P.F., and C.L. edited and revised manuscript; D.F., I.D.B., S.K., F.H., T.H., M.H., J.P.F., and C.L. approved final version of manuscript.

REFERENCES

1. **Ainslie PN, Bailey DM.** Your ageing brain: the lows and highs of cerebral metabolism. *J Physiol* 591: 1591–1592, 2013.
2. **Ainslie PN, Cotter JD, George KP, Lucas S, Murrell C, Shave R, Thomas KN, Williams MJA, Atkinson G.** Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing. *J Physiol* 586: 4005–4010, 2008.
3. **Bailey DM, Marley CJ, Brugniaux JV, Hodson D, New KJ, Ogoh S, Ainslie PN.** Elevated aerobic fitness sustained throughout the adult lifespan is associated with improved cerebral hemodynamics. *Stroke* 44: 3235–3238, 2013.
4. **Barnes JN, Taylor JL, Kluck BN, Johnson CP, Joyner MJ.** Cerebrovascular reactivity is associated with maximal aerobic capacity in healthy older adults. *J Appl Physiol (1985)* 114: 1383–1387, 2013.
5. **Brauer P, Kochs E, Werner C, Bloom M, Policare R, Pentheny S, Yonas H, Kofke WA, Schulte am Esch J.** Correlation of transcranial Doppler sonography mean flow velocity with cerebral blood flow in patients with intracranial pathology. *J Neurosurg Anesthesiol* 10: 80–85, 1998.

6. **Chen JJ, Rosas HD, Salat DH.** Age-associated reductions in cerebral blood flow are independent from regional atrophy. *Neuroimage* 55: 468–478, 2011.
7. **Demirkaya S, Uluc K, Bek S, Vural O.** Normal blood flow velocities of basal cerebral arteries decrease with advancing age: a transcranial Doppler sonography study. *Tohoku J Exp Med* 214: 145–149, 2008.
8. **Fan JL, Leiggener C, Rey F, Kayser B.** Effect of inspired CO_2 on the ventilatory response to high intensity exercise. *Respir Physiol Neurobiol* 180: 283–288, 2012.
9. **Fisher JP, Hartwich D, Seifert T, Olesen ND, McNulty CL, Nielsen HB, van Lieshout JJ, Secher NH.** Cerebral perfusion, oxygenation and metabolism during exercise in young and elderly individuals. *J Physiol* 591: 1859–1870, 2013.
10. **Fisher JP, Ogoh S, Young CN, Raven PB, Fadel PJ.** Regulation of middle cerebral artery blood velocity during dynamic exercise in humans: influence of aging. *J Appl Physiol (1985)* 105: 266–273, 2008.
11. **Fjell AM, Walhovd KB, Fennema-Notestine C, McEvoy LK, Hagler DJ, Holland D, Brewer JB, Dale AM.** One-year brain atrophy evident in healthy aging. *J Neurosci* 29: 15223–15231, 2009.
12. **Flück D, Beaudin AE, Steinback CD, Kumarpillai G, Shobha N, McCreary CR, Peca S, Smith EE, Poulin MJ.** Effects of aging on the association between cerebrovascular responses to visual stimulation, hypercapnia and arterial stiffness. *Front Physiol* 5: 49, 2014.
13. **Frassetto L, Sebastian A.** Age and systemic acid-base equilibrium: analysis of published data. *J Gerontol A Biol Sci Med Sci* 51: B91–B99, 1996.
14. **Frassetto LA, Morris RC Jr, Sebastian A.** Effect of age on blood acid-base composition in adult humans: role of age-related renal functional decline. *Am J Physiol Renal Fluid Electrolyte Physiol* 271: F1114–F1122, 1996.
15. **Galvin SD, Celi LA, Thomas KN, Clendon TR, Galvin IF, Bunton RW, Ainslie PN.** Effects of age and coronary artery disease on cerebrovascular reactivity to carbon dioxide in humans. *Anaesth Intensive Care* 38: 710–717, 2010.
16. **Giller CA, Bowman G, Dyer H, Mootz L, Krippner W, Loftus CM, Muizelaar JP.** Cerebral arterial diameters during changes in blood-pressure and carbon-dioxide during craniotomy. *Neurosurgery* 32: 737–742, 1993.
17. **Goodall S, Gonzalez-Alonso J, Ali L, Ross EZ, Romer LM.** Supraspinal fatigue after normoxic and hypoxic exercise in humans. *J Physiol* 590: 2767–2782, 2012.
18. **Harper AM, Bell RA.** The effect of metabolic acidosis and alkalosis on the blood flow through the cerebral cortex. *J Neurol Neurosurg Psychiatry* 26: 341–344, 1963.
19. **Harris AD, Ide K, Poulin MJ, Frayne R.** Control of end-tidal P_{CO_2} reduces middle cerebral artery blood velocity variability: implications for physiological neuroimaging. *Neuroimage* 29: 1272–1277, 2006.
20. **Hellstrom G, Fischer-Colbrie W, Wahlgren NG, Jogestrand T.** Carotid artery blood flow and middle cerebral artery blood flow velocity during physical exercise. *J Appl Physiol (1985)* 81: 413–418, 1996.
21. **Ide K, Eliasziw M, Poulin MJ.** Relationship between middle cerebral artery blood velocity and end-tidal P_{CO_2} in the hypocapnic-hypercapnic range in humans. *J Appl Physiol* 95: 129–137, 2003.
22. **Ito H, Kanno I, Ibaraki M, Hatazawa J.** Effect of aging on cerebral vascular response to $PaCO_2$ changes in humans as measured by positron emission tomography. *J Cereb Blood Flow Metab* 22: 997–1003, 2002.
23. **Jones NL, Robertson DG, Kane JW.** Difference between end-tidal and arterial P_{CO_2} in exercise. *J Appl Physiol* 47: 954–960, 1979.
24. **Kastrup A, Dichgans J, Niemeier M, Schabet M.** Changes of cerebrovascular CO_2 reactivity during normal aging. *Stroke* 29: 1311–1314, 1998.
25. **Kety SS.** Human cerebral blood flow and oxygen consumption as related to aging. *J Chronic Dis* 3: 478–486, 1956.
26. **Kontos HA, Raper AJ, Patterson JL.** Analysis of vasoactivity of local pH, P_{CO_2}, and bicarbonate on pial vessels. *Stroke* 8: 358–360, 1977.
27. **Liu Y, Zhu X, Feinberg D, Guenther M, Gregori J, Weiner MW, Schuff N.** Arterial spin labeling MRI study of age and gender effects on brain perfusion hemodynamics. *Magn Reson Med* 68: 912–922, 2012.
28. **Marsden KR, Haykowsky MJ, Smirl JD, Jones H, Nelson MD, Altamirano-Diaz LA, Gelinas JC, Tzeng YC, Smith KJ, Willie CK, Bailey DM, Ainslie PN.** Aging blunts hyperventilation-induced hypocapnia and reduction in cerebral blood flow velocity during maximal exercise. *Age (Dordr)* 34: 725–735, 2012.
29. **Martin AJ, Friston KJ, Colebatch JG, Frackowiak RS.** Decreases in regional cerebral blood flow with normal aging. *J Cereb Blood Flow Metab* 11: 684–689, 1991.
30. **Matteis M, Troisi E, Monaldo BC, Caltagirone C, Silvestrini M.** Age and sex differences in cerebral hemodynamics: a transcranial Doppler study. *Stroke* 29: 963–967, 1998.
31. **Miyachi M, Kawano H, Sugawara J, Takahashi K, Hayashi K, Yamazaki K, Tabata I, Tanaka H.** Unfavorable effects of resistance training on central arterial compliance: a randomized intervention study. *Circulation* 110: 2858–2863, 2004.
32. **Murrell CJ, Cotter JD, Thomas KN, Lucas SJE, Williams MJA, Ainslie PN.** Cerebral blood flow and cerebrovascular reactivity at rest and during sub-maximal exercise: effect of age and 12-week exercise training. *Age* 35: 905–920, 2012.
33. **Nielsen HB, Boushel R, Madsen P, Secher NH.** Cerebral desaturation during exercise reversed by O_2 supplementation. *Am J Physiol Heart Circ Physiol* 277: H1045–H1052, 1999.
34. **Nybo L, Rasmussen P.** Inadequate cerebral oxygen delivery and central fatigue during strenuous exercise. *Exerc Sport Sci Rev* 35: 110–118, 2007.
35. **Ogoh S, Ainslie PN.** Cerebral blood flow during exercise: mechanisms of regulation. *J Appl Physiol (1985)* 107: 1370–1380, 2009.
36. **Olin JT, Dimmen AC, Subudhi AW, Roach RC.** Cerebral blood flow and oxygenation at maximal exercise: the effect of clamping carbon dioxide. *Respir Physiol Neurobiol* 175: 176–180, 2011.
37. **Peebles K, Celi L, McGrattan K, Murrell C, Thomas K, Ainslie PN.** Human cerebrovascular and ventilatory CO2 reactivity to end-tidal, arterial and internal jugular vein P_{CO_2}. *J Physiol* 584: 347–357, 2007.
38. **Poulin MJ, Robbins PA.** Indexes of flow and cross-sectional area of the middle cerebral artery using Doppler ultrasound during hypoxia and hypercapnia in humans. *Stroke* 27: 2244–2250, 1996.
39. **Querido JS, Sheel AW.** Regulation of cerebral blood flow during exercise. *Sports Med* 37: 765–782, 2007.
40. **Rakobowchuk M, McGowan CL, de Groot PC, Bruinsma D, Hartman JW, Phillips SM, MacDonald MJ.** Effect of whole body resistance training on arterial compliance in young men. *Exp Physiol* 90: 645–651, 2005.
41. **Scharhag-Rosenberger F, Carlsohn A, Lundby C, Schuler S, Mayer F, Scharhag J.** Can more than one incremental cycling test be performed within one day? *Eur J Sport Sci* 14: 459–467, 2014.
42. **Schwertfeger N, Neu P, Schlattmann P, Lemke H, Heuser I, Bajbouj M.** Cerebrovascular reactivity over time course in healthy subjects. *J Neurol Sci* 249: 135–139, 2006.
43. **Shvartz E, Reibold RC.** Aerobic fitness norms for males and females aged 6 to 75 years: a review. *Aviat Space Environ Med* 61: 3–11, 1990.
44. **Siebenmann C, Sørensen H, Jacobs RA, Haider T, Rasmussen P, Lundby C.** Hypocapnia during hypoxic exercise and its impact on cerebral oxygenation, ventilation and maximal whole body O_2 uptake. *Respir Physiol Neurobiol* 185: 461–467, 2013.
45. **Subudhi AW, Olin JT, Dimmen AC, Polaner DM, Kayser B, Roach RC.** Does cerebral oxygen delivery limit incremental exercise performance? *J Appl Physiol* 111: 1727–1734, 2011.
46. **Thomas BP, Yezhuvath US, Tseng BY, Liu P, Levine BD, Zhang R, Lu H.** Life-long aerobic exercise preserved baseline cerebral blood flow but reduced vascular reactivity to CO_2. *J Magn Reson* 38: 1177–1183, 2013.
47. **Vianna LG, Koulouris N, Lanigan C, Moxham J.** Effect of acute hypercapnia on limb muscle contractility in humans. *J Appl Physiol* 69: 1486–1493, 1990.
48. **Yamamoto M, Meyer JS, Sakai F, Yamaguchi F.** Aging and cerebral vasodilator responses to hypercarbia: responses in normal aging and in persons with risk factors for stroke. *Arch Neurol* 37: 489–496, 1980.
49. **Zhu YS, Tarumi T, Tseng BY, Palmer DM, Levine BD, Zhang R.** Cerebral vasomotor reactivity during hypo- and hypercapnia in sedentary elderly and Masters athletes. *J Cereb Blood Flow Metab* 33: 1190–1196, 2013.
50. **Zhu YS, Tseng BY, Shibata S, Levine BD, Zhang R.** Increases in cerebrovascular impedance in older adults. *J Appl Physiol* 111: 376–381, 2011.

Cerebrovascular reactivity is increased with acclimatization to 3 454 m altitude.

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Running headline: Cerebrovascular reactivity and high altitude exposure

Abstract

Controversy exists regarding the effect of high altitude exposure on cerebrovascular CO_2 reactivity (CVR). Confounding factors in previous studies include the use of different experimental approaches, ascent profiles, duration and severity of exposure and plausibly environmental factors associated with altitude exposure. One aim of the present study was to determine CVR throughout acclimatization to high altitude when controlling for these. Middle cerebral artery mean velocity ($MCAv_{mean}$) CVR was assessed during hyperventilation (hypocapnia) and CO_2 administration (hypercapnia) with background normoxia [sea-level (SL)] and hypoxia (3 454 m) in nine healthy volunteers [26±4years (mean±SD)] at SL, and after 30 min (HA0), 3 (HA3) and 22 (HA22) days of high altitude (3 454 m) exposure.

At altitude, ventilation was increased whereas $MCAv_{mean}$ was not altered. Hypercapnic CVR was decreased at HA0 (1.16±0.16%/mmHg, mean±SEM) whereas both hyper- and hypocapnic CVR were increased at HA3 (3.13±0.18 and 2.96±0.10%/mmHg) and HA22 (3.32±0.12 and 3.24±0.14%/mmHg) compared with SL (1.98±0.22 and 2.38±0.10%/mmHg) ($p<0.01$) regardless of background oxygenation. Cerebrovascular conductance ($MCAv_{mean}$/mean arterial pressure) CVR was determined to account for blood pressure changes and revealed an attenuated response. Collectively our results demonstrate that hypocapnic and hypercapnic CVR are both elevated with acclimatization to high altitude.

Keywords: brain blood flow, cerebrovascular conductance, CO_2 reactivity, hypoxia, middle cerebral artery, TCD

Introduction

Ensuring an adequate cerebral blood flow and O_2 delivery is crucial and is in normoxia largely regulated by the arterial partial pressure of CO_2 (P_ACO_2).[1] Elevations in P_ACO_2 (hypercapnia) lead to vasodilation, whereas decreases in P_ACO_2 (hypocapnia) facilitate vasoconstriction of the cerebrovasculature.[1] The cerebrovasculature is less sensitive to changes in the arterial O_2 partial pressure (P_AO_2), with vasodilation only occurring when P_AO_2 declines below ~ 60 mmHg .[2] With acute exposure to high altitude, the hypoxic vasodilatory effect facilitates an elevation in cerebral blood flow, however with acclimatization to altitude this is counteracted by the hypoxic ventilatory response which causes a reduction in P_ACO_2 and thus cerebral blood flow declines towards sea-level values.[3, 4] Cerebrovascular CO_2 reactivity (CVR), a measure of cerebrovascular function,[5] may intuitively be expected to be decreased in response to the ventilatory induced reduction in P_ACO_2 in order to preserve cerebral blood flow. Yet, several studies have assessed CVR in response to hypoxic exposure[6-15] and the outcomes are highly controversial, ranging from increased,[7, 9, 13, 14] unchanged[10, 12, 15] to reduced[6, 8, 11] CVR. These differences possibly derive in part from inconsistencies in study protocols and methods applied. In this regard, the CVR tests have been conducted with background oxygen levels ranging from being hypoxic,[6, 10-12] normoxic[6, 10] to hyperoxic.[7-9, 11, 13] This may influence the results as peripheral chemoreceptor sensitivity is increased in response to chronic hypoxia[16] and in addition, the hypoxic vasodilatory effect may affect CVR.[2] Moreover, the time points of CVR assessment in the above mentioned studies vary not only between studies from several minutes to 16 days at hypoxia, but also within studies where measurements were conducted e.g within 2 – 4 days.[8] Finally, only a few studies have taken changes in blood pressure into account when assessing CVR,[7, 9] despite the demonstrated effects of hypoxia, hyper- and hypocapnia on blood pressure[17] and cerebral autoregulation.[18, 19] Furthermore, most studies have been conducted under conditions similar to a mountain expedition[8, 9, 11, 15] including a trekking ascent over several days, board and lodging, which contrast markedly with subject's usual environment and habits, all being potential confounding factors.

To overcome, at least in part, these limitations we performed a study at the Jungfraujoch research station (3 454 m) where living conditions are comparable to subject's daily life and allow for measurements immediately after a two hour train ascent. Hypo- and hypercapnic CVR were assessed in background hypoxia and normoxia at sea level and then again after exactly 30 min, 3 and 22 days of hypoxic exposure. We hypothesized that hypo- and hypercapnic CVR would be unaffected during acute exposure to high altitude, but would increase with acclimatization.

Materials and Methods

All experimental protocols and procedures conformed to the Declaration of Helsinki and were approved by the Ethical committee of the Swiss Federal Institute of Technology Zurich (EK 2011-N-51). Prior to participation, a detailed verbal and written explanation of the study was provided, and written informed consent to participation was obtained from each participant.

Nine young and healthy sea-level residents (1 female) with a mean age of 26 ± 4 years (mean ± SD), height of 179 ± 9 cm and weight of 75 ± 10 kg, volunteered to participate in the study. They were not taking any medication and had no history of cardiovascular, cerebrovascular or respiratory disease. Subjects refrained from sleeping at > 2 500 m within the last 3 months prior to the study. They were instructed to avoid caffeine, alcohol and exercise within 12 h prior to the experiments reported here.

Study design

Following a full familiarization trial of the experiment detailed below, volunteers underwent four experimental trials: one in Zurich, Switzerland (SL, 432 m) and three at the Jungfraujoch research station (3 454 m), one thirty minutes after a 2 hour ascent by train, one after 3 days (HA3) and the last after 22 days (HA22). Each subject travelled to the research station individually to allow testing at the same daytime throughout all experimental trials as well as after the same duration spent at high altitude.

Hypo- and hypercapnic CVR protocol

All experimental protocols were conducted with study volunteers in a semi supine position. A 10 min resting period, which included 5 min baseline data collection (BL), preceded the hypo- and hypercapnic CVR protocol. The hypo- and hypercapnic CVR protocol included four different levels of end tidal partial pressure of CO_2 ($P_{ET}CO_2$) which each conducted at two different levels

of background oxygenation (partial pressure of inspired O_2, P_IO_2) simulating 432 m (normoxia) and 3 454 m (hypoxia) in a randomized order. During the first step of the hypo- and hypercapnic protocol study volunteers were instructed to hyperventilate for 2 min in order to reduce $P_{ET}CO_2$ to 17 – 20 mmHg (HV). The second step served to normalize $P_{ET}CO_2$ (plus0). Based on this second step $P_{ET}CO_2$ was increased by 5 and 10 mmHg for the third (plus5) and fourth (plus10) step of the hypo- and hypercapnic protocol, respectively. The duration of each step was 3 min with the aim to attain at least 2 min with the desired levels of P_IO_2 and $P_{ET}CO_2$. In order to reach the desired levels of P_IO_2 and $P_{ET}CO_2$ nitrogen or oxygen and CO_2 was added to inspiration (Altitrainer, SMTEC, Nyon, Switzerland).

Experimental measures

$MCAv_{mean}$ was assessed using transcranial Doppler ultrasonography (TCD, Doppler Box, DWL, Sipplingen, Germany) with a 2 MHz probe placed over the right temporal window, prepared with ultrasound gel. The probe was held in place with a headgear. Mean arterial pressure (MAP) was recorded continuously via finger photoplethysmography (Nexfin, BMEYE B.V, Amsterdam, Netherlands) and heart rate (HR) and oxygen saturation (SpO_2) were assessed by a pulse oximetry (Nellcor Oximax N-600, Mansfield, MA, USA). $MCAv_{mean}$, MAP, HR and SpO_2 were sampled at 1000 Hz and stored for offline analysis (LabChart 7 Pro v7.3.5 and Powerlab, ADInstruments, Bella Vista, NSW, Australia).

By breathing through a mouthpiece with the nose occluded (Hans Rudolph, Kansas City, USA) respiratory parameters were measured breath-by-breath using a spirometer (Cosmed Quark b2, Rome, Italy).

Capillary blood samples were obtained from the right ear lobe at BL and at the end of all steps during the hypo- and hypercapnic CVR protocol. $P_{CAP}O_2$, $P_{CAP}CO_2$, pH and $[HCO_3^-]$ were measured using a blood gas analyzer (ABL800, Radiometer, Copenhagen, Denmark).

Cerebral tissue oxygenation ($cStO_2$) was continuously assessed on the left and right forehead by near infrared spectroscopy (NIRS; Invos-5100c, Covidien, Mansfield, MA, USA).

Data analysis

Data was recorded continuously. Values are presented as means ± SEM. Data parameters were averaged over the last min of each step during the hypo- and hypercapnic CVR protocol. Cerebrovascular conductance (CVC) was calculated as MCA v_{mean} divided by MAP.

Hypo- and hypercapnic CVR was calculated as absolute and relative changes in MCA v_{mean} and relative changes in CVC (CVCR) divided by the change in $P_{ET}CO_2$.

Comparisons of values were made using a repeated two-way ANOVA with the main factor being time (SL, HA0, HA3 and HA22) and condition (normoxia and hypoxia). Tukey's range test was applied for post hoc analysis. The Pearson product-moment correlation was used to examine the relationship between CVR and CVCR and bicarbonate. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using SAS Enterprise Guide (4.3, SAS Institute, Inc., Cary, NC, USA).

Results

All nine volunteers completed the entire study protocol at SL and high altitude.

Cardiovascular, respiratory and cerebrovascular parameters at SL, HA0, HA3 and HA22 are presented in Table 1. Briefly, HR, MAP and $MCAv_{mean}$ did not change ($p > 0.25$) in response to acute or chronic exposure to high altitude. Ventilation (VE) increased by 41 ± 7 % from SL to HA22 ($p < 0.01$) and was associated to a decline in $P_{ET}CO_2$ by 24 ± 2 % ($p < 0.01$). $P_{ET}O_2$ decreased to 54.4 ± 1.7 mmHg ($p < 0.01$) in response to high altitude exposure at HA0, but thereafter increased with prolonged exposure to high altitude compared with HA0 ($p < 0.01$).

Hypo- and hypercapnic CVR protocol

Figure 1 illustrates $P_{ET}CO_2$ and P_IO_2 during the hypo- and hypercapnic CVR protocols. In background normoxia (Figure 1A) P_IO_2 values were 138 ± 0.5 mmHg and in hypoxia (Figure 1B) 94.8 ± 0.3 mmHg. During hyperventilation $P_{ET}CO_2$ values decreased to 18.7 ± 0.1 mmHg (Figure 1A and 1B).

$MCAv_{mean}$, MAP, CVC and VE during hypo- and hypercapnic CVR test

Percent changes in hypo- and hypercapnic CVR are presented in Figure 2. CVR conducted in either a normoxic or hypoxic background was elevated at HA3 and HA22 compared with SL and HA0 ($p < 0.01$). Overall percent changes in hypo- and hypercapnic CVR were higher with a normoxic background compared with the hypoxic background (2.33 ± 0.11 vs. 2.09 ± 0.11 %/mmHg, $p < 0.01$). Similar changes were assessed as absolute changes in CVR in response to hypo- and hypercapnia. From SL to HA22 absolute changes in hypocapnic CVR tested in normoxia and hypoxia were elevated from 1.50 ± 0.14 and 1.34 ± 0.06 cm/s/mmHg to 2.07 ± 0.17 and 1.70 ± 0.13 cm/s/mmHg ($p < 0.01$), respectively. Absolute changes in CVR to hypercapnia at HA0 were

reduced compared with SL (0.68 ± 0.10 vs. 1.26 ± 0.15 cm/s/mmHg, $p < 0.01$) and thereafter increased ($p < 0.01$) compared with SL and HA0.

MAP decreased in response to hypocapnia and increased during hypercapnia ($p < 0.01$, Table 2). CVCR values are presented in Figure 3. In response to hypocapnia CVCR was elevated following HA22 compared with SL independent of the background level of oxygen ($p < 0.01$). A trend was observed within the CVCR values in normoxic and hypoxic background ($p = 0.07$). Additionally there was a trend for a reduction in hypercapnic CVCR from SL to HA0 ($p = 0.07$).

Ventilation during the hypercapnic CVR protocol was increased with exposure to high altitude (e.g. SL_{plus10}, 24.1 ± 3.1; $HA22_{plus10}$, 38.8 ± 4.4 l/min; $p < 0.01$) as well as with elevated $P_{ET}CO_2$ in background normoxia (e.g. SL_{plus0}, 7.1 ± 0.4; SL_{plus10}, 24.1 ± 3.1 l/min; $p < 0.01$) and hypoxia (e.g. SL_{plus0}, 8.4 ± 0.6; SL_{plus10}, 19.6 ± 2.5 l/min; $p < 0.01$). During the hypocapnic CVR protocol ventilation decreased with exposure to high altitude independent of background oxygen levels (e.g. SL_{HV}, 61.1 ± 7.2, $HA22_{HV}$, 34.3 ± 2.6 l/min; $p < 0.01$).

Capillary blood samples

$P_{CAP}O_2$ and $P_{CAP}CO_2$ followed the same pattern as $P_{ET}O_2$ and $P_{ET}CO_2$, an initial decrease followed by an increase and a decline throughout the exposure to high altitude, respectively (Table 1). Bicarbonate was decreased at HA3 and HA22 ($p < 0.01$, Table 1) compared with SL and HA0.

CVR and bicarbonate correlations

Hypercapnic CVR correlated with the bicarbonate values in response high altitude ($p < 0.01$, Figure 4) independent of background oxygen levels.

Discussion

In this study, we determined the effect of high altitude (3 454 m) exposure on CVR in a controlled setting where confounding factors were minimized. Our findings extend those from previous studies by demonstrating (1) an initial decline in hypercapnic CVR observed 30 min after high altitude exposure which was followed by (2) an enhanced hypercapnic CVR at HA3 and HA22 in background normoxia and hypoxia. Furthermore, (3) hypocapnic CVR was increased with acclimatization and (4) when accounting for changes in blood pressure this led to attenuated hyper- and hypocapnic CVRs.

Elevated CBF has been commonly reported within the first days at high altitude due to the vasodilatory effect of hypoxia.[3, 8] Thereafter, CBF is reported to decrease towards baseline in response to the hypoxic ventilatory response causing a reduction in $P_{ET}CO_2$ and thus leading to vasoconstriction.[3, 4, 8] In the present study, $MCAv_{mean}$ did not increase following the first 30 min of exposure to high altitude, despite a drop in $P_{ET}O_2$ (Table 1) below the presumed vasodilatory threshold.[2] This is in agreement with other studies[6, 11, 20] and likely related to the fact that $P_{ET}O_2$ was only slightly below the vasodilatory threshold while the simultaneous decline in $P_{ET}CO_2$ counteracted the vasodilatory effect. On the other hand, previous studies have assessed $MCAv_{mean}$ using TCD as also the case in the current study. This technique may have underestimated the changes in CBF given that hypoxia may increase vessel diameter.[21]

Controversial results exist on the effect of high altitude exposure on cerebrovascular function. In the present study we observed a decreased hypercapnic CVR at HA0 followed by an increased hypercapnic CVR at HA3 and HA22 compared with SL. In most previous studies acute measurements were not feasible due to the limited accessibility to the high altitude research facilities. In an attempt to overcome this limitation, Fan et al.[7] supplied their volunteers with supplemental oxygen during the 3 h ascent from 1 525 to 5 260 m. However, volunteers flew to 4 000 m and descended to 1 525 m for 48 h before ascending to 5 260 m, and thus they were not assessed in strictly acute altitude conditions. Consequently, this may have led to the increased CVR compared with SL, which contrasts the results of the present study. In agreement with the present

results is the reported reduction in CVR in response to normobaric hypoxia in a controlled laboratory setting.[6] Other studies have noted decreased CVRs but following 2 – 15 days of exposure to high altitude.[8, 10] A proposed mechanistic explanation here fore is enhanced sympathetic activation. However increased sympathetic nerve activity with hypoxia is not limiting for further dilation of larger extracranial blood vessels.[22] On the other hand, the decrease in CVR during acute exposure to high altitude may prevent the already elevated pH to increase even further, by limiting the washout of hydrogen ions.[23] This would support the unchanged $MCAv_{mean}$ at HA0. With acclimatization to high altitude the increase in pH is counterbalanced by the renal acid-base compensation, manifested by a reduction in $[HCO_3^-]$,[24] which has been suggested to be linked to the increased CVR following 2 - 4 and 16 days sojourn at high altitude.[7, 9, 14] This supports the results of the present study (Figure 2) together with hypercapnic CVR being correlated to changes in $[HCO_3^-]$ with acclimatization (Figure 4).

Studies assessing CVR have not all distinguished between hypo- and hypercapnic reactivity. The most recent study has estimated a $MCAv_{mean}$-CO_2 slope including the hypo- and hypercapnic range,[7] although the effect of altitude on cerebrovascular responses has been suggested to differ between the hypo- and hypercapnic range[8, 10] as demonstrated in the current study. Fewer discrepancies in response to high altitude have been reported in the hypocapnic range. Increased hypocapnic reactivity at HA3 and HA22 in the present study is in line with previous findings.[8, 9]

Background oxygen level during CVR tests is a further methodological consideration yielding different CVR results. In the present study normoxic and hypoxic background levels during the CVR tests were chosen, simulating SL and 3 454 m oxygen levels, respectively, resulting in similar responses to exposure to high altitude. This is in agreement with Fan et al.[7] also demonstrating an enhanced CVR with acclimatization to high altitude, when however assessed in a hyperoxic background. Thus background oxygen level seems not to have an effect on CVR in response to exposure to high altitude.

Changes in $P_{ET}CO_2$ may lead to changes in blood pressure and thus affect CVR.[25, 26] Additionally alterations in cerebral autoregulation in response to hypercapnia[27] and hypoxia[6, 28, 29] have been

reported. To overcome the potential influence of MAP we have monitored MAP (Table 2) continuously throughout the experiments and calculated CVCR (Figure 3) to reveal direct effects of changes in MAP on CVR as it has been suggested.[17] In comparison to CVR, CVCR resulted in attenuated responses to high altitude acclimatization, decreased hypercapnic CVR at H0 and increased hypocapnic CVR at HA3 disappeared.

A limitation of the study is the use of TCD to assess $MCAv_{mean}$, a surrogate for CBF. $MCAv_{mean}$ is a measure of blood flow velocity and not a flow in absolute terms. Nevertheless the two have been shown to be highly correlated.[30] The majority of studies have illustrated that the diameter of the MCA does not change in response to hypo- and hypercapnia.[31, 32] Dilation of the MCA in hypoxic conditions has been demonstrated in altitudes above 5 000 m,[21, 33, 34] therefore diameter changes at 3 454 m are less likely.

In conclusion the present study represents a controlled high altitude study and extends previous findings by demonstrating an elevated hypo- and hypercapnic CVR with acclimatization to high altitude.

Disclosure/Conflict of Interest

No conflicts of interest, financial or otherwise, are declared by the author(s).

References

1. Ide K, Eliasziw M, Poulin MJ. Relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans. *J Appl Physiol* 2003; 95(1)**:** 129-37.

2. Gupta AK, Menon DK, Czosnyka M, Smielewski P, Jones JG. Thresholds for hypoxic cerebral vasodilation in volunteers. *Anesth Analg* 1997; 85(4)**:** 817-20.

3. Severinghaus JW, Chiodi H, Eger EI, Brandstater B, Hornbein TF. Cerebral blood flow in man at high altitude. Role of cerebrospinal fluid pH in normalization of flow in chronic hypocapnia. *Circ Res* 1966; 19(2)**:** 274-82.

4. Møller K, Paulson OB, Hornbein TF, Colier WN, Paulson AS, Roach RC *et al.* Unchanged cerebral blood flow and oxidative metabolism after acclimatization to high altitude. *J Cereb Blood Flow Metab* 2002; 22(1)**:** 118-26.

5. Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW *et al.* Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *J Neurosci Methods* 2011; 196(2)**:** 221-37.

6. Ogoh S, Nakahara H, Ueda S, Okazaki K, Shibasaki M, Subudhi AW *et al.* Effects of acute hypoxia on cerebrovascular responses to carbon dioxide. *Exp Physiol* 2014; 99(6)**:** 849-58.

7. Fan JL, Subudhi AW, Evero O, Bourdillon N, Kayser B, Lovering AT *et al.* AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO2 with high-altitude acclimatization and reexposure. *J Appl Physiol* 2014; 116(7)**:** 911-8.

8. Lucas SJ, Burgess KR, Thomas KN, Donnelly J, Peebles KC, Lucas RA *et al.* Alterations in cerebral blood flow and cerebrovascular reactivity during 14 days at 5050 m. *J Physiol* 2011; 589(3)**:** 741-53.

9. Fan JL, Burgess KR, Basnyat R, Thomas KN, Peebles KC, Lucas SJ *et al.* Influence of high altitude on cerebrovascular and ventilatory responsiveness to CO2. *J Physiol* 2010; 588(3)**:** 539-49.

10. Rupp T, Esteve F, Bouzat P, Lundby C, Perrey S, Levy P *et al.* Cerebral hemodynamic and ventilatory responses to hypoxia, hypercapnia, and hypocapnia during 5 days at 4,350 m. *J Cereb Blood Flow Metab* 2014; 34(1)**:** 52-60.

11. Ainslie PN, Burgess KR. Cardiorespiratory and cerebrovascular responses to hyperoxic and hypoxic rebreathing: effects of acclimatization to high altitude. *Respir Physiol Neurobiol* 2008; 161(2)**:** 201-9.

12. Villien M, Bouzat P, Rupp T, Robach P, Lamalle L, Tropres I *et al.* Changes in cerebral blood flow and vasoreactivity to CO2 measured by arterial spin labeling after 6days at 4350m. *Neuroimage* 2013; 72**:** 272-9.

13. Poulin MJ, Fatemian M, Tansley JG, O'Connor DF, Robbins PA. Changes in cerebral blood flow during and after 48 h of both isocapnic and poikilocapnic hypoxia in humans. *Exp Physiol* 2002; 87(5)**:** 633-42.

14. Jensen JB, Sperling B, Severinghaus JW, Lassen NA. Augmented hypoxic cerebral vasodilation in men during 5 days at 3,810 m altitude. *J Appl Physiol* 1996; 80(4)**:** 1214-8.

15. Jansen GF, Krins A, Basnyat B. Cerebral vasomotor reactivity at high altitude in humans. *J Appl Physiol* 1999; 86(2)**:** 681-6.

16. Duffin J. Measuring the ventilatory response to hypoxia. *J Physiol* 2007; 584(1)**:** 285-93.

17. Claassen JA, Zhang R, Fu Q, Witkowski S, Levine BD. Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *J Appl Physiol* 2007; 102(3)**:** 870-7.

18. Ainslie PN, Duffin J. Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. *Am J Physiol Regul Integr Comp Physiol* 2009; 296(5)**:** R1473-R1495.

19. Ogoh S, Nakahara H, Ainslie PN, Miyamoto T. The effect of oxygen on dynamic cerebral autoregulation: critical role of hypocapnia. *J Appl Physiol* 2010; 108(3)**:** 538-43.

20. Subudhi AW, Fan JL, Evero O, Bourdillon N, Kayser B, Julian CG *et al.* AltitudeOmics: effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery. *Exp Physiol* 2014; 99(5)**:** 772-81.

21. Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND *et al.* Regional brain blood flow in man during acute changes in arterial blood gases. *J Physiol* 2012; 590(14)**:** 3261-75.

22. Lewis NC, Messinger L, Monteleone B, Ainslie PN. Effect of acute hypoxia on regional cerebral blood flow: effect of sympathetic nerve activity. *J Appl Physiol* 2014; 116(9)**:** 1189-96.

23. Foster GE. High on altitude: new attitudes toward human cerebral blood flow regulation and altitude acclimatization. *J Physiol* 2011; 589(3)**:** 449.

24. Crawford RD, Severinghaus JW. CSF pH and ventilatory acclimatization to altitude. *J Appl Physiol* 1978; 45(2)**:** 275-83.

25. Panerai RB, Evans DH, Naylor AR. Influence of arterial blood pressure on cerebrovascular reactivity. *Stroke* 1999; 30(6)**:** 1293-5.

26. Regan RE, Fisher JA, Duffin J. Factors affecting the determination of cerebrovascular reactivity. *Brain Behav* 2014; 4(5)**:** 775-88.

27. Zhang R, Zuckerman JH, Giller CA, Levine BD. Transfer function analysis of dynamic cerebral autoregulation in humans. *Am J Physiol* 1998; 274(1)**:** H233-41.

28. Ainslie PN, Ashmead JC, Ide K, Morgan BJ, Poulin MJ. Differential responses to CO2 and sympathetic stimulation in the cerebral and femoral circulations in humans. *J Physiol* 2005; 566(2)**:** 613-24.

29. Subudhi AW, Panerai RB, Roach RC. Acute hypoxia impairs dynamic cerebral autoregulation: results from two independent techniques. *J Appl Physiol* 2009; 107(4)**:** 1165-71.

30. Brauer P, Kochs E, Werner C, Bloom M, Policare R, Pentheny S *et al.* Correlation of transcranial Doppler sonography mean flow velocity with cerebral blood flow in patients with intracranial pathology. *J Neurosurg Anesthesiol* 1998; 10(2)**:** 80-5.

31. Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 2000; 31(7)**:** 1672-8.

32. Verbree J, Bronzwaer AS, Ghariq E, Versluis MJ, Daemen MJ, van Buchem MA *et al.* Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI. *J Appl Physiol* 2014; 117(10)**:** 1084-9.

33. Willie CK, Smith KJ, Day TA, Ray LA, Lewis NC, Bakker A *et al.* Regional cerebral blood flow in humans at high altitude: gradual ascent and 2 wk at 5,050 m. *J Appl Physiol* 2014; 116(7)**:** 905-10.

34. Wilson MH, Edsell ME, Davagnanam I, Hirani SP, Martin DS, Levett DZ *et al.* Cerebral artery dilatation maintains cerebral oxygenation at extreme altitude and in acute hypoxia--an ultrasound and MRI study. *J Cereb Blood Flow Metab* 2011; 31(10)**:** 2019-29.

Table1. Cardiovascular, respiratory, cerebrovascular and capillary parameters at sea level (SL), 30 min (HA0), 3 days (HA3) and 22 days (HA22) following ascent to altitude (3 454 m).

	SL	3454 m			*P* value
		HA0	HA3	HA22	
HR (bpm)	69±5	75±5	71±3	70±4	0.53
MAP (mmHg)	93.0±4.2	94.3±3.0	99.5±4.7	95.8±4.6	0.25
VE (l/min)	9.5±0.6	10.4±0.4	11.2±0.73*	13.3±0.8*†‡	< 0.01
BF (1/min)	15.2±1.4	17.0±1.5	17.4±1.1	18.1±1.5	0.08
V_T (l)	0.67±0.07	0.67±0.09	0.67±0.07	0.79±0.09	0.28
$P_{ET}O_2$ (mmHg)	91.5±1.1	54.4±1.7*	58.0±0.8*	64.2±1.2*†‡	< 0.01
$P_{ET}CO_2$ (mmHg)	39.5±0.5	37.1±0.9*	32.3±0.5*†	30.0±0.6*†‡	< 0.01
SpO_2 (%)	97.2±0.6	88.5±1.8*	89.5±0.9*	93.1±0.5†	< 0.01
MCA v_{mean} (cm/s)	61.2±3.4	62.0±3.9	59.5±3.7	58.2±2.9	0.68
CVC (cm/s/mmHg)	0.67±0.05	0.67±0.05	0.61±0.05	0.62±0.04	0.14
$cStO_2$ (%)	75.6±2.5	64.1±2.4*	69.0±3.3*	71.9±2.1†	< 0.01
pH	7.38±0.01	7.40±0.01	7.40±0.01	7.39±0.01	0.06
$P_{CAP}CO_2$ (mmHg)	38.7±0.8	36.5±1.2	32.0±0.6*†	30.8±0.6*†	< 0.01
$P_{CAP}O_2$ (mmHg)	72.0±1.8	50.6±1.7*	55.3±1.1*	59.8±1.1*†	< 0.01
$[HCO_3^-]$ (mmol/l)	22.6±0.3	22.6±0.2	21.1±0.5*†	20.4±0.4*†	< 0.01

Values are mean±SEM. HR, heart rate; MAP, mean arterial pressure; VE, ventilation; BF, breathing frequency; V_T, tidal volume; $P_{ET}O_2$, end-tidal partial pressure of O_2; $P_{ET}CO_2$, end-tidal partial pressure of CO_2; SpO_2, oxygen saturation; MCA v_{mean}, middle cerebral artery mean velocity; CVC, cerebrovascular conductance; $cStO_2$, cerebral tissue oxygenation; $P_{CAP}CO_2$, capillary partial pressure of CO_2; $P_{CAP}O_2$, capillary partial pressure of O_2; $[HCO_3^-]$, bicarbonate concentration. *P* values represent ANOVA results. * $P < 0.05$ vs SL. † $p < 0.05$ vs HA0. ‡ $p < 0.05$ vs HA3.

Table 2. Mean arterial pressure (MAP) during hypocapnic and hypercapnic testing at sea level (SL), 30 min (HA0), 3 days (HA3) and 22 days (HA22) following ascent to high altitude (3 454 m).

		Normoxia				Hypoxia				*P* values
		HV	Plus0	Plus5	Plus10	HV	Plus0	Plus5	Plus10	
MAP	SL	92.6±3.3	94.8±3.8	97.1±3.8	99.5±3.7	93.3±3.0	95.7±3.2	97.1±3.8	99.5±3.6	Condition p=0.042
(mmHg)	HA0	90.9±3.3	95.5±2.7	97.7±2.6	99.6±2.9	95.1±3.1	98.9±3.2	98.6±2.5	99.7±2.6	Time p<0.001
	HA3	97.0±3.0	102.2±3.4	103.1±3.3	106.9±3.3	96.2±2.3	103.5±2.4	103.1±3.3	106.3±3.1	Test p<0.001
	HA22	92.7±3.3	97.0±3.0	101.2±3.0	104.9±3.8	95.7±3.7	100.9±3.9	101.2±3.0	108.8±3.9	

Values are mean±SEM. MAP, mean arterial pressure. P values represent ANOVA results. Condition, Normoxia vs. Hypoxia; Time, SL, HA0, HA3 and HA22; Test, HV, Plus0, Plus5 and Plus10.

Titles and legends to figures

Figure 1. End tidal partial pressure of CO_2 ($P_{ET}CO_2$, dashed lines) and inspired partial pressure of O_2 (P_IO_2; solid lines) during hyperventilation (HV), no additional CO_2 (plus0), plus 5 mmHg CO_2 (plus5) and plus 10 mmHg CO_2 (plus10) are presented in near sea level (A) and 3 454 m (B) conditions conducted at sea level (circle), 30 min (square), 3 days (diamond) and 22 days (triangle) after ascent to high altitude.

Figure 2. Individual (grey) and mean (black) $MCAv_{mean}$ percent change cerebrovascular CO_2 reactivity (CVR) in response to hypocapnic (A,C) and hypercapnia (B,D) in background normoxia (A,B) and hypoxia (C,D) at sea level (SL), 30 min (HA0), 3 days (HA3) and 22 days (HA22) after ascent to high altitude . *P* values represent ANOVA results. * $p < 0.05$ vs SL. † $p < 0.05$ vs HA0.

Figure 3. Individual (grey) and mean (black) cerebrovascular conductance percent change cerebrovascular CO_2 reactivity (CVCR) in response to hypocapnic (A,C) and hypercapnia (B,D) in background normoxia (A,B) and hypoxia (C,D) at sea level (SL), 30 min (HA0), 3 days (HA3) and 22 days (HA22) after ascent to high altitude. *P* values represent ANOVA results. * $p < 0.05$ vs SL. † $p < 0.05$ vs HA0.

Figure 4. Relation between hypercpanic $MCAv_{mean}$ cerebrovascular CO_2 reactivity (CVR) (A, B) and cerebrovascular conductance CO_2 reactivity (CVCR) (C, D) percent changes and bicarbonate in background normoxia (A, C) and hypoxia (B, D) at sea level (black circles), 30 min (dark gray squares), 3 days (gray diamonds) and 22 days (white triangles) upon ascent to high altitude.

Figure 1

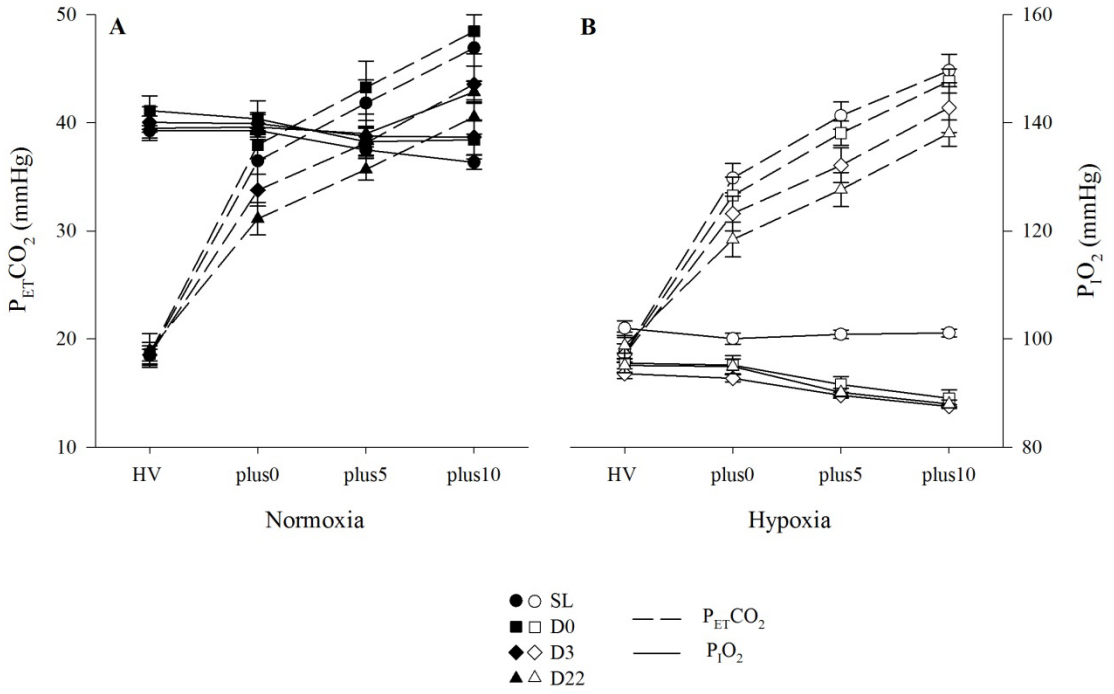

A
B
50
40
30
20
10
160
140
120
100
80
$P_{ET}CO_2$ (mmHg)
P_IO_2 (mmHg)
HV
plus0
plus5
plus10
Normoxia
Hypoxia
SL
D0
D3
D22
$P_{ET}CO_2$
P_IO_2

Figure 2

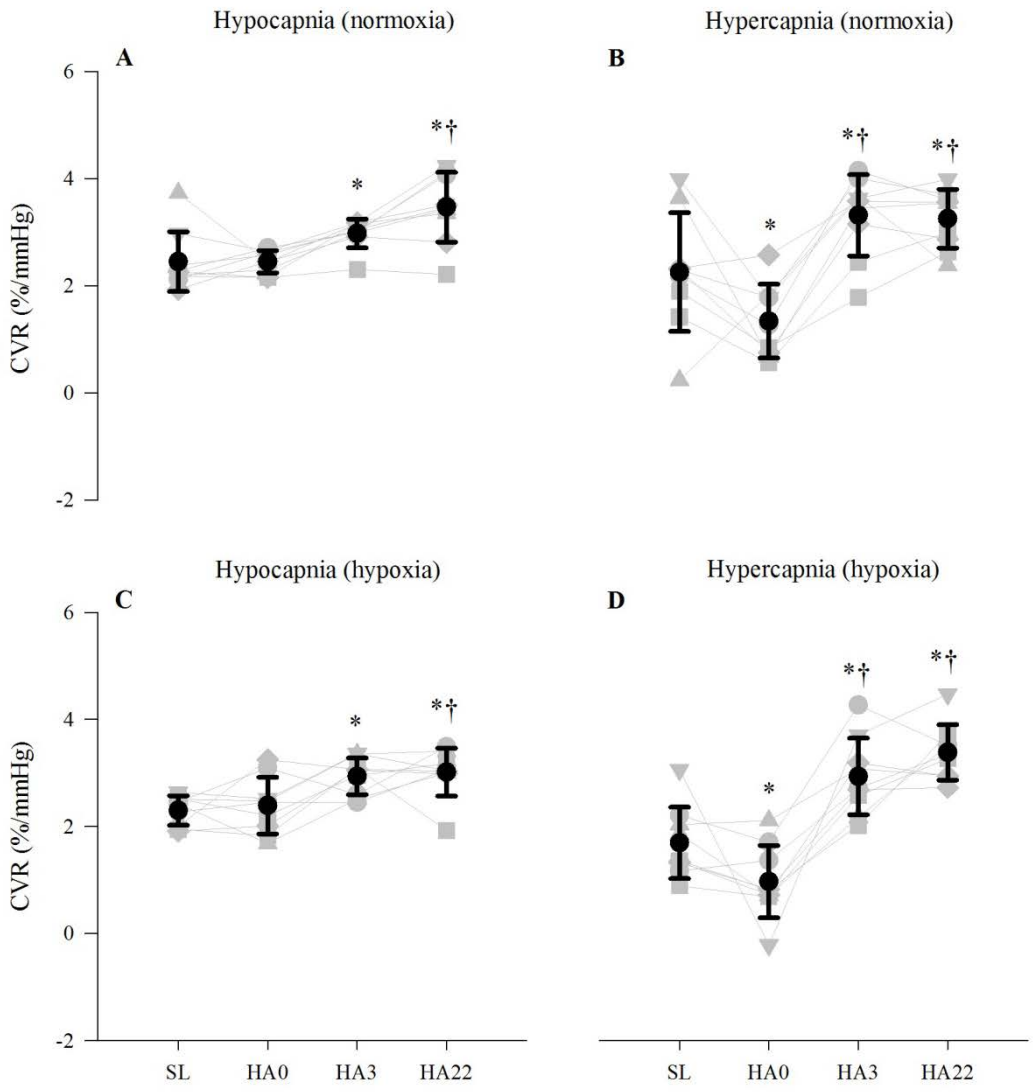
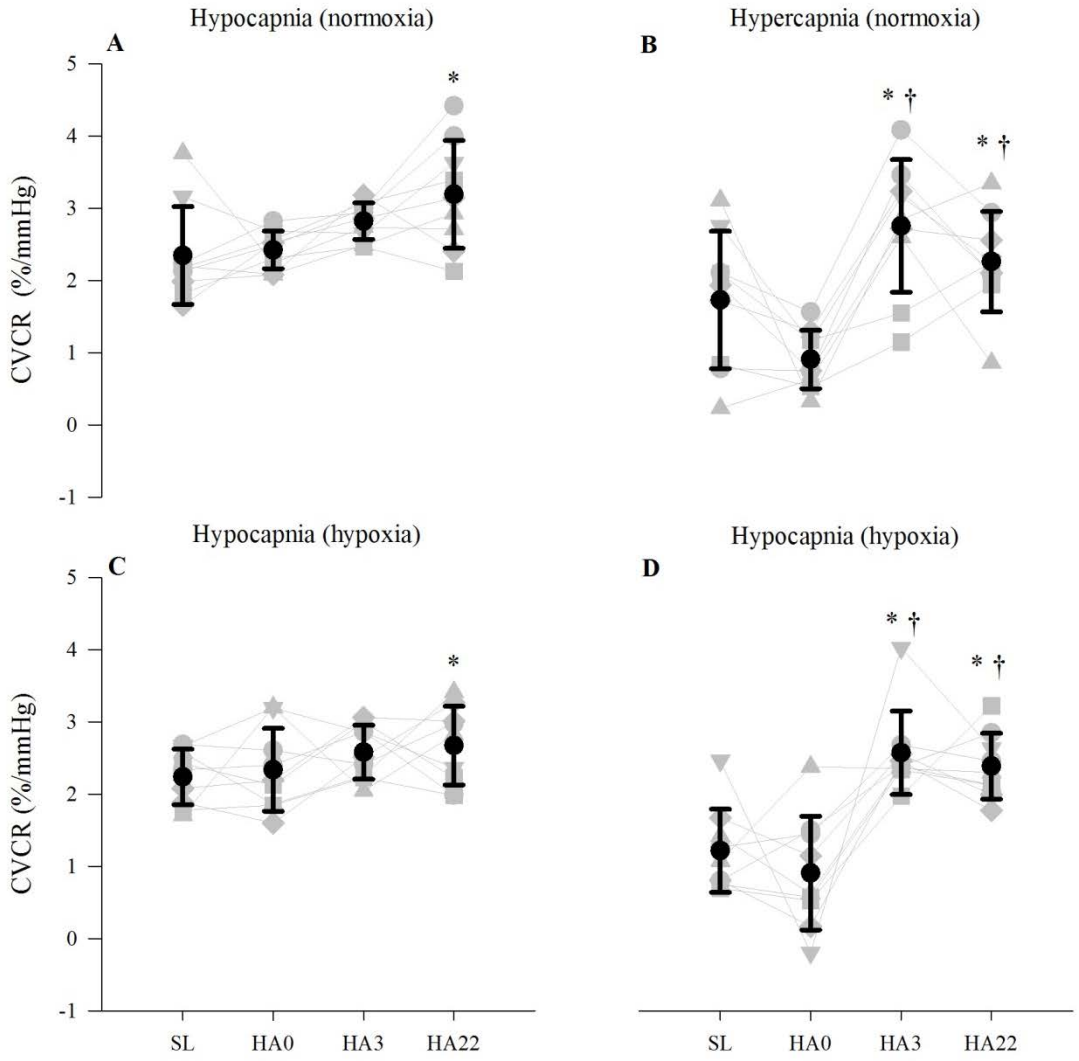

Hypocapnia (normoxia)
A
Hypercapnia (normoxia)
B
Hypocapnia (hypoxia)
C
Hypercapnia (hypoxia)
D
CVR (%/mmHg)
6
4
2
0
-2
*
*†
SL
HA0
HA3
HA22

Figure 3


Hypocapnia (normoxia)
A
Hypercapnia (normoxia)
B
Hypocapnia (hypoxia)
C
Hypocapnia (hypoxia)
D
CVCR (%/mmHg)
5
4
3
2
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SL
HA0
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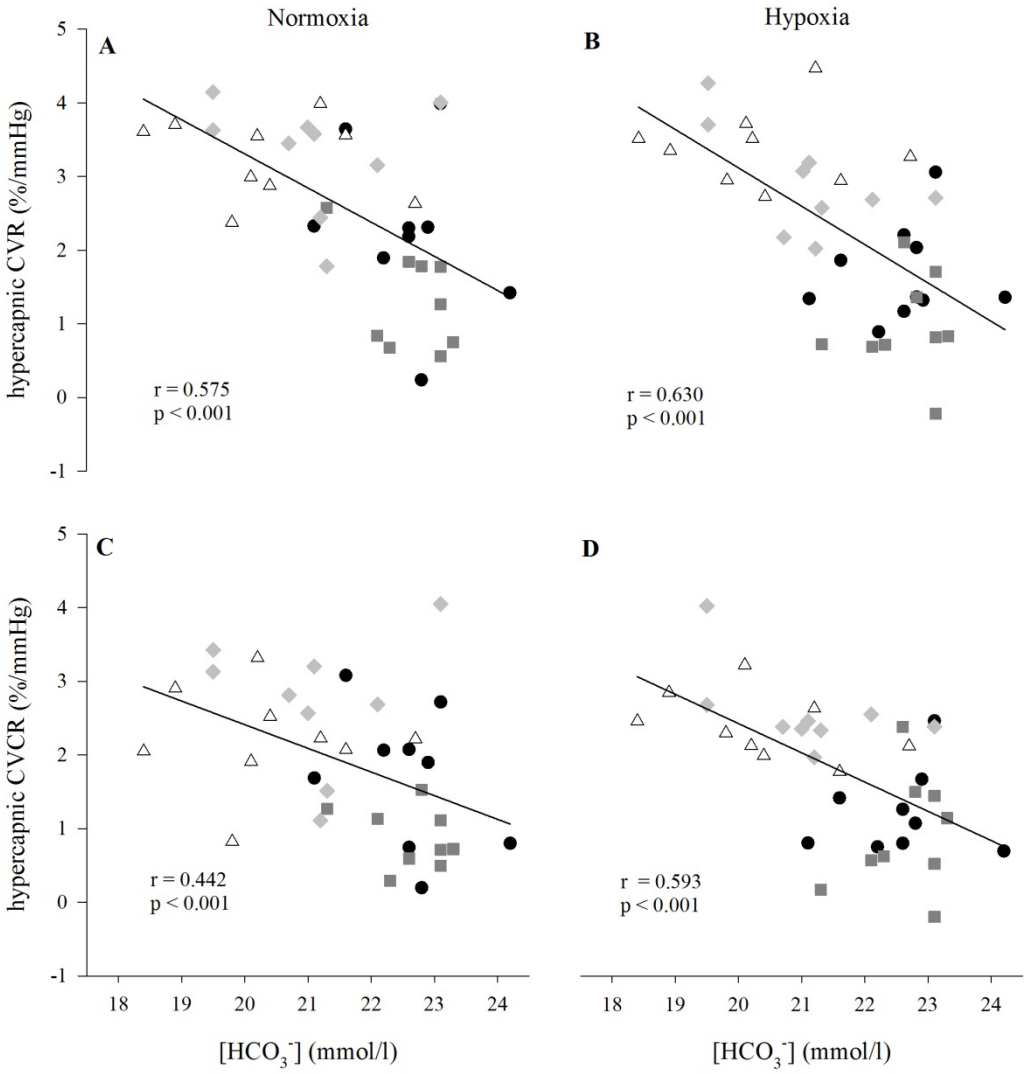
Figure 4


Normoxia
Hypoxia
A
B
C
D
hypercapnic CVR (%/mmHg)
hypercapnic CVCR (%/mmHg)
r = 0.575
p < 0.001
r = 0.630
p < 0.001
r = 0.442
p < 0.001
r = 0.593
p < 0.001
[HCO3-] (mmol/l)
[HCO3-] (mmol/l)

4. Conclusion and outlook

The purpose of the present PhD project was to 1) elucidate the role of CO_2 on the regulation of CBF during exercise with aging. To better understand alterations in CBF due to changes in CO_2 with aging and during exercise, CO_2 was added to inspiration during exercise in young and older volunteers; and 2) to study the influence of high altitude on CVR. For this purpose, hypercapnic and hypocapnic CVR were assessed in young and healthy volunteers during acute and prolonged exposure to high altitude.

As the results obtained in each study are discussed in detail in the included manuscripts, this section summarizes the key points and highlights future directions for research within the corresponding fields.

4.1 Age, aerobic fitness, and cerebral perfusion during exercise: Role of carbon dioxide

CBF is attenuated with increasing age, both at rest and during exercise (Kety 1956; Ainslie et al. 2008; Fisher et al. 2012) and concomitantly lower $PaCO_2$ values have been observed in elderly (Fisher et al. 2012). Cerebrovascular function has also been demonstrated to be reduced in elderly (Ito et al. 2002; Flück et al. 2014). To investigate the underlying mechanisms of the age-associated reduction in CBF, CVR was assessed in young and older study volunteers and supplemental CO_2 was administered to the inspiration during exercise to examine the influence of the age-dependent reduction in $PaCO_2$. Additional aims included investigating the effect of aerobic fitness on the decline in CBF with aging and examining whether decreased CBF and thus cerebral oxygenation at maximal exercise limits exercise performance.

In agreement with previous studies (Kety 1956; Ainslie et al. 2008; Fisher et al. 2012) CBF was reduced at rest and during exercise with aging. CVR was however not reduced in the present study, and hence seems not to contribute to the age-related reduction in CBF. In contrast, administration of CO_2 to inspiration during exercise abolished the age-related decrease in CBF by ≈ 50 % and thus the observed reduction in $PaCO_2$ with age seems to explain most of the reduction in CBF.

Previously, aerobic fitness has been associated with a diminished decline in CBF with increasing age (Ainslie et al. 2008). However, higher aerobic capacity in our young and older study participants did not lead to improved CVR. Finally, although the drop in CBF with increasing exercise intensity was prevented by adding CO_2 to inspiration and thus maintained cerebral oxygenation, this however did not lead to an increased exercise performance. Thus, indicating decreased cerebral oxygenation not to be a limiting factor for exercise performance.

4.2 Cerebrovascular reactivity increases with acclimatization to 3454 m

Controversy exists regarding the effect of high altitude exposure on CVR, as increases, decreases or no changes have been observed. Confounding factors in previous studies include the use of different experimental approaches, ascent profiles, duration and severity of exposure and plausibly environmental factors associated with altitude exposure. The aim of the present study was to determine hypercapnic and hypocapnic in response to acute and throughout acclimatization to high altitude when controlling for these.

At the Jungfraujoch research station (3454 m), where confounding factors are minimized, hypercapnia and hypocapnic CVR were assessed in background normoxia and hypoxia. Our data extend those from previous studies by demonstrating an initial decline in hypercapnic CVR observed after 30 min of high altitude exposure, which was followed by an enhanced hypercapnic CVR after 3 and 22 days at high altitude independent of background oxygenation. Furthermore, hypocapnic CVR was increased with acclimatization. Accounting for changes in blood pressure led to attenuated hyper- and hypocapnic CVRs. This underlines the importance of controlling for confounding factors. Overall, the cerebrovasculature maintains or even improves its reactivity in response to prolonged exposure to high altitude, however, whether this is beneficial for altitude acclimatization needs further investigation.

4.3 Outlook

As reduced brain perfusion is associated with cognitive impairment and brain damage, it is of general interest to prevent the decline in CBF with increasing age. The present PhD project revealed a decreased $PaCO_2$ to be partly responsible for the age-related decline in CBF during

exercise. The origin of the observed hypocapnia at rest and during exercise in the older individuals is unknown. A reduced CO_2 production, hyperventilation, decreased pulmonary perfusion, metabolic acidosis (Frassetto and Sebastian 1996; Laffey and Kavanagh 2002) are all possible mechanisms leading to hypocapnia. Hyperventilation, however, can be ruled out in the present study, as ventilation did not differ between young and old individuals. Further investigations are needed to elucidate the underlying mechanisms of hypocapnia in older individuals and its effect on the cerebrovasculature. Secondly, CBF underlies an integrative regulation and besides $PaCO_2$ being a major regulator of CBF other factors contribute to the regulation of CBF. Additional research is required to fully understand age-associated effects on CBF during exercise. Sympathetic nerve activity, cerebral autoregulation and cerebrovascular health may all play a role. A possible way to investigate the influence of sympathetic nerve activity on the age-related reduction in CBF during exercise is to specifically inhibit the sympathetic nervous system.

Increased cerebrovascular reactivity is associated with increased cerebrovascular health. In the present PhD project an elevated cerebrovascular reactivity to hyper- and hypocapnia was observed with acclimatization to high altitude. Intuitively, this would mean an increased cerebrovascular health and therefore this may serve to maintain precise regulation of CBF in this environment. On the other hand, as $PaCO_2$ decreases with exposure to high altitude and hypocapnic reactivity has been shown to be increased this leads to a proportionally greater reduction in cerebral perfusion. Whether an increased reactivity to hyper- and hypocapnia of the cerebrovasculature at altitude benefits or even prevents high altitude sickness remains to be further investigated.

Furthermore, underlying mechanisms leading to altered CVR in response to acute and chronic exposure to high altitude are still uncertain. Administration of bicarbonate during altitude exposure could clarify the role of acid-base compensation on the increase in hypercapnic CVR. Additionally, as $PaCO_2$ is decreased in response to high altitude exposure and further reduced by the hypoxic ventilatory response, a different stimulus might reveal less biased information on the reactivity of the cerebrovasculature at high altitude. This could e.g. be a flashing checkerboard (visual

stimulation), which affects blood flow to the visual cortex and thus regional cerebrovascular reactivity could be assessed in the posterior cerebral artery.

In addition, technological advances allow measurement of CBF with a higher accuracy by measuring internal carotid and vertebral arteries volumetrically using linear-array vascular ultrasound. Additionally, transcranial color coded duplex technology enables to assess changes in diameter of the MCA (Willie et al. 2012; Willie et al. 2014) and thus overcomes the flow versus velocity limitation.

From a personal point of view, I have been fortunate to receive support from the Swiss National Science Foundation to join Prof. Dr. Philip Ainslie's laboratory at the University of British Columbia, Kelowna, Canada for a post doc fellowship. This enables me to not only acquire the new techniques within the field but also to further elucidate the regulation of CBF and its underlying mechanisms. Specifically, I will focus on sympathetic nerve activity and cerebral metabolism and their role in the regulation of CBF in response to changes in MAP.

5. Bibliography

Aaslid, R., T. M. Markwalder and H. Nornes (1982). "Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries." J Neurosurg 57(6): 769-774.

Ainslie, P. N., J. C. Ashmead, K. Ide, B. J. Morgan and M. J. Poulin (2005). "Differential responses to CO2 and sympathetic stimulation in the cerebral and femoral circulations in humans." J Physiol 566(2): 613-624.

Ainslie, P. N. and K. R. Burgess (2008). "Cardiorespiratory and cerebrovascular responses to hyperoxic and hypoxic rebreathing: effects of acclimatization to high altitude." Respir Physiol Neurobiol 161(2): 201-209.

Ainslie, P. N., J. D. Cotter, K. P. George, S. Lucas, C. Murrell, R. Shave, K. N. Thomas, M. J. A. Williams and G. Atkinson (2008). "Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing." J Physiol 586(16): 4005-4010.

Ainslie, P. N. and J. Duffin (2009). "Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation." Am J Physiol Regul Integr Comp Physiol 296(5): R1473-R1495.

Apkon, M., R. A. Weed and W. F. Boron (1997). "Motor responses of cultured rat cerebral vascular smooth muscle cells to intra- and extracellular pH changes." Am J Physiol 273(1 Pt 2): H434-445.

Bailey, D. M., C. J. Marley, J. V. Brugniaux, D. Hodson, K. J. New, S. Ogoh and P. N. Ainslie (2013). "Elevated aerobic fitness sustained throughout the adult lifespan is associated with improved cerebral hemodynamics." Stroke 44(11): 3235-3238.

Bakker, S. L., F. E. de Leeuw, T. den Heijer, P. J. Koudstaal, A. Hofman and M. M. Breteler (2004). "Cerebral haemodynamics in the elderly: the rotterdam study." Neuroepidemiology 23(4): 178-184.

Barnes, J. N., J. L. Taylor, B. N. Kluck, C. P. Johnson and M. J. Joyner (2013). "Cerebrovascular reactivity is associated with maximal aerobic capacity in healthy older adults." J Appl Physiol 114(10): 1383-1387.

Belanger, M., I. Allaman and P. J. Magistretti (2011). "Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation." Cell Metab 14(6): 724-738.

Berne, R. M., H. R. Winn and R. Rubio (1981). "The local regulation of cerebral blood flow." Prog Cardiovasc Dis 24(3): 243-260.

Bertsch, K., D. Hagemann, M. Hermes, C. Walter, R. Khan and E. Naumann (2009). "Resting cerebral blood flow, attention, and aging." Brain Res 1267: 77-88.

Boushel, R., H. Langberg, J. Olesen, J. Gonzales-Alonzo, J. Bulow and M. Kjaer (2001). "Monitoring tissue oxygen availability with near infrared spectroscopy (NIRS) in health and disease." Scand J Med Sci Sports 11(4): 213-222.

Brown, A. M. and B. R. Ransom (2007). "Astrocyte glycogen and brain energy metabolism." Glia 55(12): 1263-1271.

Chen, J. J., H. D. Rosas and D. H. Salat (2011). "Age-associated reductions in cerebral blood flow are independent from regional atrophy." Neuroimage 55(2): 468-478.

Chesler, M. (2003). "Regulation and modulation of pH in the brain." Physiol Rev 83(4): 1183-1221.

Cipolla, M. J. (2009). The cerebral circulation. Integrated Systems Physiology: From Molecule to Function. San Rafael (CA), Morgan & Claypool Life Sciences.

Claassen, J. A., R. Zhang, Q. Fu, S. Witkowski and B. D. Levine (2007). "Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing." J Appl Physiol 102(3): 870-877.

Demirkaya, S., K. Uluc, S. Bek and O. Vural (2008). "Normal blood flow velocities of basal cerebral arteries decrease with advancing age: a transcranial Doppler sonography study." Tohoku J Exp Med 214(2): 145-149.

Fan, J. L., K. R. Burgess, R. Basnyat, K. N. Thomas, K. C. Peebles, S. J. Lucas, R. A. Lucas, J. Donnelly, J. D. Cotter and P. N. Ainslie (2010). "Influence of high altitude on cerebrovascular and ventilatory responsiveness to CO2." J Physiol 588(3): 539-549.

Fan, J. L., A. W. Subudhi, O. Evero, N. Bourdillon, B. Kayser, A. T. Lovering and R. C. Roach (2014). "AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO2 with high-altitude acclimatization and reexposure." J Appl Physiol 116(7): 911-918.

Fick, A. (1870). "Ueber die Messung des Blutquantums in den Herzventrikeln." Phys Med Ges Würzburg Bd II:XVI.

Fisher, J. P., D. Hartwich, T. Seifert, N. D. Olesen, C. L. McNulty, H. B. Nielsen, J. J. van Lieshout and N. H. Secher (2012). "Cerebral perfusion, oxygenation and metabolism during exercise in young and elderly individuals." J Physiol 591(7): 1859-1870.

Fisher, J. P., S. Ogoh, C. N. Young, P. B. Raven and P. J. Fadel (2008). "Regulation of middle cerebral artery blood velocity during dynamic exercise in humans: influence of aging." J Appl Physiol 105(1): 266-273.

Fjell, A. M., K. B. Walhovd, C. Fennema-Notestine, L. K. McEvoy, D. J. Hagler, D. Holland, J. B. Brewer and A. M. Dale (2009). "One-Year Brain Atrophy Evident in Healthy Aging." J Neurosci 29(48): 15223-15231.

Flück, D., A. E. Beaudin, C. D. Steinback, G. Kumarpillai, N. Shobha, C. R. McCreary, S. Peca, E. E. Smith and M. J. Poulin (2014). "Effects of aging on the association between cerebrovascular responses to visual stimulation, hypercapnia and arterial stiffness." Front Physiol 5: 49.

Frassetto, L. and A. Sebastian (1996). "Age and systemic acid-base equilibrium: analysis of published data." J Gerontol A Biol Sci Med Sci 51(1): B91-99.

Galvin, S. D., L. A. Celi, K. N. Thomas, T. R. Clendon, I. F. Galvin, R. W. Bunton and P. N. Ainslie (2010). "Effects of age and coronary artery disease on cerebrovascular reactivity to carbon dioxide in humans." Anaesth Intensive Care 38(4): 710-717.

Goodall, S., J. Gonzalez-Alonso, L. Ali, E. Z. Ross and L. M. Romer (2012). "Supraspinal fatigue after normoxic and hypoxic exercise in humans." J Physiol 590(Pt 11): 2767-2782.

Gupta, A. K., D. K. Menon, M. Czosnyka, P. Smielewski and J. G. Jones (1997). "Thresholds for hypoxic cerebral vasodilation in volunteers." Anesth Analg 85(4): 817-820.

Hamner, J. W., C. O. Tan, K. Lee, M. A. Cohen and J. A. Taylor (2010). "Sympathetic control of the cerebral vasculature in humans." Stroke 41(1): 102-109.

Harper, A. M. and R. A. Bell (1963). "The Effect of Metabolic Acidosis and Alkalosis on the Blood Flow through the Cerebral Cortex." J Neurol Neurosurg Psychiatry 26: 341-344.

Hellström, G., W. Fischer-Colbrie, N. G. Wahlgren and T. Jogestrand (1996). "Carotid artery blood flow and middle cerebral artery blood flow velocity during physical exercise." J Appl Physiol 81(1): 413-418.

Iadecola, C. (1992). "Does nitric oxide mediate the increases in cerebral blood flow elicited by hypercapnia?" Proc Natl Acad Sci U S A 89(9): 3913-3916.

Ide, K., M. Eliasziw and M. J. Poulin (2003). "Relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans." J Appl Physiol 95(1): 129-137.

Ito, H., I. Kanno, M. Ibaraki and J. Hatazawa (2002). "Effect of aging on cerebral vascular response to PaCO2 changes in humans as measured by positron emission tomography." J Cereb Blood Flow Metab 22(8): 997-1003.

Jansen, G. F., A. Krins and B. Basnyat (1999). "Cerebral vasomotor reactivity at high altitude in humans." J Appl Physiol 86(2): 681-686.

Jensen, J. B., B. Sperling, J. W. Severinghaus and N. A. Lassen (1996). "Augmented hypoxic cerebral vasodilation in men during 5 days at 3,810 m altitude." J Appl Physiol 80(4): 1214-1218.

Jones, N. L., D. G. Robertson and J. W. Kane (1979). "Difference between end-tidal and arterial PCO2 in exercise." J Appl Physiol Respir Environ Exerc Physiol 47(5): 954-960.

Kety, S. S. (1956). "Human cerebral blood flow and oxygen consumption as related to aging." J Chronic Dis 3(5): 478-486.

Kety, S. S. and C. F. Schmidt (1948). "The Effects of Altered Arterial Tensions of Carbon Dioxide and Oxygen on Cerebral Blood Flow and Cerebral Oxygen Consumption of Normal Young Men." J Clin Invest 27(4): 484-492.

Kety, S. S. and C. F. Schmidt (1948). "The Nitrous Oxide Method for the Quantitative Determination of Cerebral Blood Flow in Man: Theory, Procedure and Normal Values." J Clin Invest 27(4): 476-483.

Kontos, H. A., A. J. Raper and J. L. Patterson (1977). "Analysis of vasoactivity of local pH, PCO2 and bicarbonate on pial vessels." Stroke 8(3): 358-360.

Laffey, J. G. and B. P. Kavanagh (2002). "Hypocapnia." N Engl J Med 347(1): 43-53.

Lassen, N. A. (1959). "Cerebral blood flow and oxygen consumption in man." Physiol Rev 39(2): 183-238.

Liu, Y., X. Zhu, D. Feinberg, M. Guenther, J. Gregori, M. W. Weiner and N. Schuff (2012). "Arterial spin labeling MRI study of age and gender effects on brain perfusion hemodynamics." Magn Reson Med 68(3): 912-922.

Lucas, S. J., K. R. Burgess, K. N. Thomas, J. Donnelly, K. C. Peebles, R. A. Lucas, J. L. Fan, J. D. Cotter, R. Basnyat and P. N. Ainslie (2011). "Alterations in cerebral blood flow and cerebrovascular reactivity during 14 days at 5050 m." J Physiol 589(3): 741-753.

Marsden, K. R., M. J. Haykowsky, J. D. Smirl, H. Jones, M. D. Nelson, L. A. Altamirano-Diaz, J. C. Gelinas, Y. C. Tzeng, K. J. Smith, C. K. Willie, D. M. Bailey and P. N. Ainslie (2012). "Aging blunts hyperventilation-induced hypocapnia and reduction in cerebral blood flow velocity during maximal exercise." Age (Dordr) 34(3): 725-735.

Melamed, E., S. Lavy, S. Bentin, G. Cooper and Y. Rinot (1980). "Reduction in regional cerebral blood flow during normal aging in man." Stroke 11(1): 31-35.

Møller, K., O. B. Paulson, T. F. Hornbein, W. N. Colier, A. S. Paulson, R. C. Roach, S. Holm and G. M. Knudsen (2002). "Unchanged cerebral blood flow and oxidative metabolism after acclimatization to high altitude." J Cereb Blood Flow Metab 22(1): 118-126.

Murrell, C. J., J. D. Cotter, K. N. Thomas, S. J. E. Lucas, M. J. A. Williams and P. N. Ainslie (2012). "Cerebral blood flow and cerebrovascular reactivity at rest and during sub-maximal exercise: Effect of age and 12-week exercise training." Age 35(3): 905-920.

Nielsen, H. B., R. Boushel, P. Madsen and N. H. Secher (1999). "Cerebral desaturation during exercise reversed by O2 supplementation." Am J Physiol 277(3 Pt 2): H1045-1052.

Ogoh, S. and P. N. Ainslie (2009). "Cerebral blood flow during exercise: mechanisms of regulation." J Appl Physiol 107(5): 1370-1380.

Ogoh, S. and P. N. Ainslie (2009). "Regulatory mechanisms of cerebral blood flow during exercise: new concepts." Exerc Sport Sci Rev 37(3): 123-129.

Ogoh, S., H. Nakahara, S. Ueda, K. Okazaki, M. Shibasaki, A. W. Subudhi and T. Miyamoto (2014). "Effects of acute hypoxia on cerebrovascular responses to carbon dioxide." Exp Physiol 99(6): 849-858.

Olin, J. T., A. C. Dimmen, A. W. Subudhi and R. C. Roach (2011). "Cerebral blood flow and oxygenation at maximal exercise: the effect of clamping carbon dioxide." Respir Physiol Neurobiol 175(1): 176-180.

Peebles, K., L. Celi, K. McGrattan, C. Murrell, K. Thomas and P. N. Ainslie (2007). "Human cerebrovascular and ventilatory CO2 reactivity to end-tidal, arterial and internal jugular vein PCO2." J Physiol 584(Pt 1): 347-357.

Pires, P. W., C. M. Dams Ramos, N. Matin and A. M. Dorrance (2013). "The effects of hypertension on the cerebral circulation." Am J Physiol Heart Circ Physiol 304(12): H1598-1614.

Poulin, M. J., M. Fatemian, J. G. Tansley, D. F. O'Connor and P. A. Robbins (2002). "Changes in cerebral blood flow during and after 48 h of both isocapnic and poikilocapnic hypoxia in humans." Exp Physiol 87(5): 633-642.

Poulin, M. J., P. J. Liang and P. A. Robbins (1996). "Dynamics of the cerebral blood flow response to step changes in end-tidal PCO2 and PO2 in humans." J Appl Physiol 81(3): 1084-1095.

Poulin, M. J. and P. A. Robbins (1996). "Indexes of flow and cross-sectional area of the middle cerebral artery using Doppler ultrasound during hypoxia and hypercapnia in humans." Stroke 27(12): 2244-2250.

Querido, J. S. and A. W. Sheel (2007). "Regulation of cerebral blood flow during exercise." Sports Med 37(9): 765-782.

Regan, R. E., J. A. Fisher and J. Duffin (2014). "Factors affecting the determination of cerebrovascular reactivity." Brain Behav 4(5): 775-788.

Rupp, T., F. Esteve, P. Bouzat, C. Lundby, S. Perrey, P. Levy, P. Robach and S. Verges (2014). "Cerebral hemodynamic and ventilatory responses to hypoxia, hypercapnia, and hypocapnia during 5 days at 4,350 m." J Cereb Blood Flow Metab 34(1): 52-60.

Serrador, J. M., P. A. Picot, B. K. Rutt, J. K. Shoemaker and R. L. Bondar (2000). "MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis." Stroke 31(7): 1672-1678.

Severinghaus, J. W., H. Chiodi, E. I. Eger, B. Brandstater and T. F. Hornbein (1966). "Cerebral blood flow in man at high altitude. Role of cerebrospinal fluid pH in normalization of flow in chronic hypocapnia." Circ Res 19(2): 274-282.

Siebenmann, C., H. Sørensen, R. A. Jacobs, T. Haider, P. Rasmussen and C. Lundby (2013). "Hypocapnia during hypoxic exercise and its impact on cerebral oxygenation, ventilation and maximal whole body O2 uptake." Respir Physiol Neurobiol 185(2): 461-467.

Thomas, B. P., U. S. Yezhuvath, B. Y. Tseng, P. Liu, B. D. Levine, R. Zhang and H. Lu (2013). "Life-long aerobic exercise preserved baseline cerebral blood flow but reduced vascular reactivity to CO2." J Magn Reson 38(5): 1177-1183.

Van Lieshout, J. J., W. Wieling, J. M. Karemaker and N. H. Secher (2003). "Syncope, cerebral perfusion, and oxygenation." J Appl Physiol 94(3): 833-848.

Verbree, J., A. S. Bronzwaer, E. Ghariq, M. J. Versluis, M. J. Daemen, M. A. van Buchem, A. Dahan, J. J. van Lieshout and M. J. van Osch (2014). "Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI." J Appl Physiol 117(10): 1084-1089.

Villien, M., P. Bouzat, T. Rupp, P. Robach, L. Lamalle, I. Tropres, F. Esteve, A. Krainik, P. Levy, J. M. Warnking and S. Verges (2013). "Changes in cerebral blood flow and vasoreactivity to CO2 measured by arterial spin labeling after 6days at 4350m." Neuroimage 72: 272-279.

Willie, C. K., F. L. Colino, D. M. Bailey, Y. C. Tzeng, G. Binsted, L. W. Jones, M. J. Haykowsky, J. Bellapart, S. Ogoh, K. J. Smith, J. D. Smirl, T. A. Day, S. J. Lucas, L. K. Eller and P. N. Ainslie (2011). "Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function." J Neurosci Methods 196(2): 221-237.

Willie, C. K., D. B. Macleod, A. D. Shaw, K. J. Smith, Y. C. Tzeng, N. D. Eves, K. Ikeda, J. Graham, N. C. Lewis, T. A. Day and P. N. Ainslie (2012). "Regional brain blood flow in man during acute changes in arterial blood gases." J Physiol 590(14): 3261-3275.

Willie, C. K., K. J. Smith, T. A. Day, L. A. Ray, N. C. Lewis, A. Bakker, D. B. Macleod and P. N. Ainslie (2014). "Regional cerebral blood flow in humans at high altitude: gradual ascent and 2 wk at 5,050 m." J Appl Physiol 116(7): 905-910.

Willie, C. K., Y. C. Tzeng, J. A. Fisher and P. N. Ainslie (2014). "Integrative regulation of human brain blood flow." J Physiol 592(Pt 5): 841-859.

Wilson, M. H., M. E. Edsell, I. Davagnanam, S. P. Hirani, D. S. Martin, D. Z. Levett, J. S. Thornton, X. Golay, L. Strycharczuk, S. P. Newman, H. E. Montgomery, M. P. Grocott and C. H. Imray (2011). "Cerebral artery dilatation maintains cerebral oxygenation at extreme altitude and in acute hypoxia--an ultrasound and MRI study." J Cereb Blood Flow Metab 31(10): 2019-2029.

Zhu, Y.-S., T. Tarumi, B. Y. Tseng, D. M. Palmer, B. D. Levine and R. Zhang (2013). "Cerebral vasomotor reactivity during hypo- and hypercapnia in sedentary elderly and Masters athletes." J Cereb Blood Flow Metab 33(8): 1190-1196.

Zhu, Y. S., B. Y. Tseng, S. Shibata, B. D. Levine and R. Zhang (2011). "Increases in cerebrovascular impedance in older adults." J Appl Physiol 111(2): 376-381.